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Studies of Influenza in Hospitals of the British Armies in France, 1918

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MEDICAL RESEARCH COMMITTEE

Studies of Influenza in Hospitals of the British Armies in France, 1918

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INTRODUCTION

During the pandemic of influenza in 1918, of which two waves appeared in the spring and autumn respectively, the Committee were able to arrange, so far as war conditions allowed, that the researches into the disease undertaken by civilian workers should be brought into relation to work being done at the same time for the Navy, the Army, and for the Air Force, and for this they were greatly indebted to the courtesy and active encouragement of the Directors of those Medical Services. The free interchange of opinions and the correlation of work done in so many different directions, both at home and overseas, have greatly aided the efforts towards better knowledge of this destructive infection and its results.

The Committee are now further indebted to the Army Medical Service for the transmission to them of the several reports here presented and for permission to publish them. It is hoped that the results of parallel work done elsewhere may follow in due course; some of these are appearing already in various scientific journals, and others the Committee will hope to present at an early date.

Of the present report the greater part consists in descriptive accounts of the bacteriology and pathology of the epidemic disease as observed among the Forces in France, and these records when collated with others are likely to have increasing value as our present imperfect knowledge of the true nature of the infective process increases, and gives the clue to the proper interpretation of the recorded external phenomena.

In the first part of the report are given the results of an inquiry by a group of workers at Abbeville, organized by Colonel S. L. Cummins, C.B., C.M.G., Adviser in Pathology to the British Armies in France, the members of which undertook, in addition to the descriptive study of the disease, an experimental investigation of its causation. The Committee are specially indebted to Colonel Cummins not only for allowing them to be kept in touch with the progress of the work, and for the introductory account of it he has written here, but also for the editorial supervision of this report in its various parts.

The work at Abbeville, which suggests that the primary infective agent in influenza is a filter-passing virus, distinct from the *Bacillus influenzae*, was unfortunately interrupted at too early a stage. Major H. G. Gibson, R.A.M.C., who was leader of the investigating team, succumbed himself to the disease when gravely overworked not only by his labours during the epidemic, but by unremitting and distinguished service given throughout the war. After the Armistice, moreover, military exigencies soon led to the stoppage of work in the Abbeville laboratories. The results of the

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work done up to that point, however, of which a preliminary account was given in the *British Medical Journal* of December 14, 1918, are here presented by Major Gibson's colleagues. As is well known, similar results as to the experimental transmission of the disease in animals by a filter-passing virus were being obtained quite independently at the same time by workers in another Army laboratory at Etaples, and these have been recently published.[1] It is plainly of the first importance that both sets of inquiries should be repeated by other hands, and that confirmation of their findings should be sought in every direction.

[1] Bradford, Wilson, and Bashford, *Quarterly Journal of Experimental Medicine*, 1919, **12**, 259.

MEDICAL RESEARCH COMMITTEE,
15 Buckingham Street,
Strand,
W.C. 2.

June 30, 1919.

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INTRODUCTION.

By Colonel S. L. CUMMINS, C.M.G., A.M.S.

On the reappearance of influenza in epidemic form in October 1918, the officers-in-charge of laboratories throughout the British Expeditionary Force in France were encouraged to pay the closest attention to the disease and to report all findings of interest.

At the same time a ' Research Team ' was organized at Abbeville in order that bacteriologists, pathologists, and clinicians might co-operate in the investigation of the epidemic. The bacteriological and pathological work was entrusted to Major H. Graeme Gibson, R.A.M.C. (secretary to the Research Team), Major F. B. Bowman, C.A.M.C., Captain A. T. Nankivell, R.A.M.C., and Captain J. I. Connor, A.A.M.C. Captain A. T. Nankivell was moved to another station in December 1918, after which Captain J. I. Connor took sole charge of the work on morbid histology. The clinical work was entrusted to Major C. E. Sundell, R.A.M.C., officer-in-charge of the medical division of No. 2 Stationary Hospital. He was assisted, during some weeks, by Captain G. E. Beaumont, R.A.M.C., who, however, had to be transferred elsewhere in the course of military duty before the work was completed.

The research work on the rôle of a filtrable virus in influenza was rendered possible by the generous help of the Medical Research Committee. The provision of monkeys was a matter of no small difficulty, but these were promptly obtained and dispatched to France without delay through the kindness of the secretary, Sir Walter Fletcher, K.B.E., F.R.S., who was, at all times, ready to

further the research by every means in his power. Major-General Sir John Rose Bradford, K.C.M.G., C.B., F.R.S., the consultant physician to the Abbeville Area, kindly attended some of the meetings of the 'Research Team', and his assistance and advice were always at the disposal of the clinicians taking part in the work.

The work of the officers-in-charge of laboratories throughout the B.E.F. was beyond praise, and many reports of high value were received. It is regretted that considerations of space impose limits on the number of these reports which it is possible to publish at the present time, but they constitute a collection of records which will be of permanent value for army reference.

The present report is divided into two parts.

Part I consists of the work contributed by the Research Team, together with diagrams, photographs, and coloured illustrations, the latter by Sergeant A. K. Maxwell, R.A.M.C., whose services were kindly lent for the purpose by the Medical Research Committee.

Part II includes clinical, pathological, bacteriological, and serological reports from No. 3 Canadian General Hospital, No. 5 General Hospital, No. 25 Stationary Hospital, and from the Central Mortuary, Etaples Base.

In addition to the reports included in Part II, papers of the highest interest were also received from Major W. James Wilson, R.A.M.C., of No. 54 General Hospital, Captain C. Ponder, R.A.M.C., of No. 1 Stationary Hospital, Lieutenant B. A. I. Peters, R.A.M.C., of No. 14 General Hospital, Captains G. Bradbury and E. B. Krumbhaar, Medical Corps, U.S. Army, of No. 16 (Philadelphia, U.S.A.) General Hospital, and from Captain H. M. Perry, R.A.M.C., of No. 14 Stationary Hospital. Many valuable notes also reached me in private letters from Lieutenant-Colonel C. J. Martin, C.M.G., F.R.S., Major J. W. McNee, D.S.O., Captain A. Stokes, D.S.O., Captain J. Cruickshank, R.A.M.C., Major A. W. M. Ellis, C.A.M.C., and others.

The perusal of these reports and communications, as well as frequent visits to the laboratories in the B.E.F., has given me an exceptional opportunity of keeping in touch with the investigations undertaken in France, and this must be my excuse for attempting to summarize the impressions gained from a centralized study of reports from a large number of independent workers.

I. Identity of Summer and Autumn Epidemics. Epidemiological Notes.

In the *B. M. J.* of November 23, 1918, Captain M. Greenwood, R.A.M.C., sets out in a striking manner the characters of the curves of incidence in past influenza epidemics. In this paper it is stated that 'the fundamental characteristics of a primary epidemic of influenza are a very high attack rate and an approximately symmetrical distribution in time': . . . 'the graph of the epidemic is an almost symmetrical curve; the fatality is low, rarely more than 1 per cent. of the cases.' 'A secondary epidemic affects a relatively small proportion of the population, is slower in reaching its maximum, and, thereafter, declines slowly and irregularly—more slowly than it increases; its distribution is asymmetrical, and there is less con-

centration around the maximum. Further, a secondary epidemic is characterized by a vastly higher fatality than a primary epidemic.'

Through the kindness of the D.G.M.S., British Armies in France, and of Captain A. H. Greg, O.B.E., R.A.M.C., statistical officer at the head-quarters of the D.M.S., L. of C., B.E.F., I have been enabled to prepare charts of incidence of the summer and autumn epidemics as they affected the British Expeditionary Force. As in Captain M. Greenwood's curves, the abscissae are weeks, the ordinates, expressed in terms of the number of cases recorded in the worst week, show the incidence on the British armies in France. In the absence of accurate figures as to the case mortality in the summer epidemic, I have charted the total mortality per month, from all diseases, throughout the year. This brings out clearly the very slight effect of the summer epidemic upon the mortality from disease, while the marked and terrible influence of the autumn epidemic is well seen.

The actual number of cases and, in the autumn epidemic, the case mortality, are shown in Tables I and II. It should be noted that, on the first appearance of the disease in May and June, many physicians preferred to use the non-committal description ' Pyrexia of Uncertain Origin ' (P.U.O.) rather than the diagnosis ' Influenza '. Table I therefore includes the cases admitted to hospital under both headings.

In the autumn epidemic many cases were diagnosed ' Broncho-pneumonia ' owing to the onset of this complication during the attack. The incidence under both headings is seen in Table II, but the totals have been used in compiling Chart I.

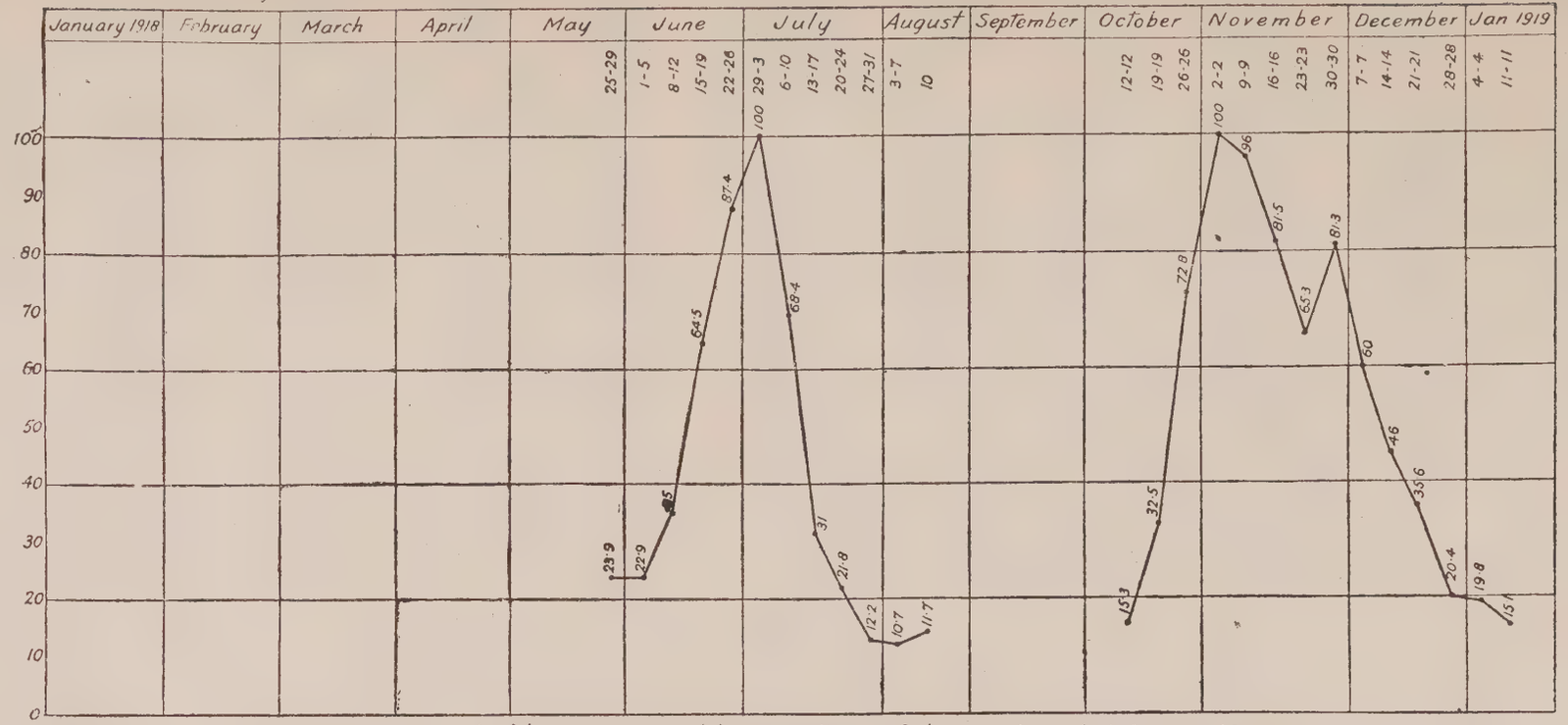
From these tables and charts it will be at once apparent that the summer and autumn epidemics exhibited, in every particular, the characters cited by Captain M. Greenwood as typical of a primary and a secondary epidemic of influenza respectively.

There can be no doubt that the two epidemics were identical in nature. The difference observed between the cases admitted in the summer and those seen in the autumn were differences in degree and depended on the enormously increased virulence of the infective agent in the secondary wave.

II. Immunity from previous Attacks.

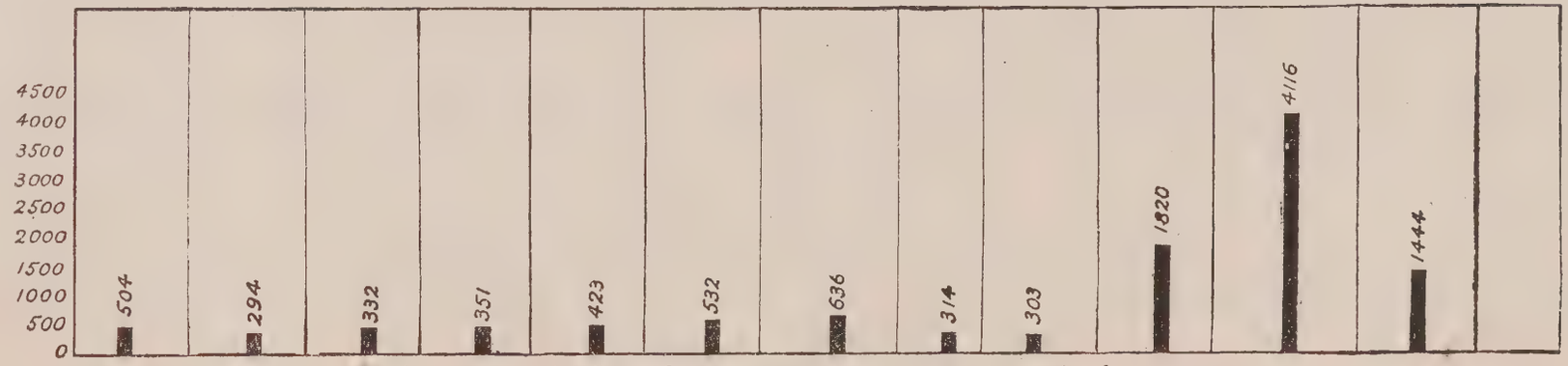
A question of great interest is whether those attacked during the summer outbreak retained any appreciable immunity in the autumn. No conclusive figures could be collected on this point owing to the errors necessarily introduced by the unequal proportion of replacements of personnel in units and formations during the intervening months. Some units, engaged in the severe fighting of the late summer and autumn, must have changed their whole personnel during the period under review, while others, such as L. of C. units, probably underwent but little change. The question of diagnosis also introduces difficulty, as common colds and slight attacks of simple pyrexia tend to be called influenza during epidemics of this disease, and it is by no means certain that a patient stating that he had had a previous attack had really suffered from the disease.

Weekly Incidence Curves of Summer and Autumn Influenza Epidemics. B.E.F. France.



Incidence expressed in percentages of the worst week taken as 100.

Total Deaths per month from Disease in Base Hospitals and C.C. Stations.



Shows marked effect of Autumn Epidemic on Total Deaths from Disease.

CHART I.

Table I.

Weekly Admissions.

Epidemic of Influenza (including Cases diagnosed P.U.O.). From Week ending May 25, 1918, to Week ending August 10, 1918.

B.E.F., FRANCE.

Week ending.	25.5.18.	1.6.18.	8.6.18.	15.6.18	22.6.18.	29.6.18.	6.7.18.	13.7.18.	20.7.18.	27.7.18.	3.8.18.	10.8.18.	*Total.*
	11,001	10,624	16,186	29,880	40,471	46,275	31,693	14,344	10,086	5,675	4,966	5,414	226,615

Table II.

Epidemic of Influenza (including Cases diagnosed Broncho-pneumonia). Total Admissions and Deaths. From Week ending October 12, 1918, to Week ending January 18, 1919.

B.E.F., FRANCE.

Week ending	*Influenza.*		*Broncho-pneumonia.*		*Total.*		*Week's case mortality.*	*Totals to date.*		*Case mortality to date.*
	New cases.	*Deaths.*	*New cases.*	*Deaths.*	*New cases.*	*Deaths.*		*New cases.*	*Deaths.*	
1918										
Oct. 12	1,665	1	225	41	1,890	42	2·2	1,890	42	2·2
Oct. 19	3,636	19	384	105	4,020	124	3·1	5,910	166	2·8
Oct. 26	8,287	93	693	258	8,980	351	3·9	14,890	517	3·5
Nov. 2	11,306	308	1,029	457	12,335	765	6·2	27,225	1,282	4·7
Nov. 9	10,846	507	1,006	484	11,852	991	8·4	39,077	2,273	5·8
Nov. 16	9,407	421	748	412	10,155	833	8·2	49,232	3,106	6·3
Nov. 23	7,339	337	720	344	8,059	681	8·5	57,291	3,787	6·6
Nov. 30	9,400	327	634	274	10,034	601	6·0	67,325	4,388	6·5
Dec. 7	6,920	248	485	222	7,405	470	6·3	74,730	4,858	6·5
Dec. 14	5,308	136	363	129	5,671	265	4·7	80,401	5,123	6·4
Dec. 21	4,178	81	219	78	4,397	159	3·6	84,798	5,282	6·2
Dec. 28	2,404	48	121	47	2,525	95	3·8	87,323	5,377	6·2
1919										
Jan. 4	2,348	28	100	28	2,448	56	2·3	89,771	5,433	6·1
Jan. 11	1,767	30	97	40	1,864	70	3·8	91,635	5,503	6·0
Jan. 18	1,925	29	110	23	2,035	52	2·6	93,670	5,555	5·9

From records at my disposal it would seem that out of 965 patients of the autumn outbreak questioned as to a previous attack, only 248, or about 26 per cent., stated that they had had influenza during the summer ; and a considerable number of these had had the disease so mildly that they were not sent to hospital. It is definitely stated by Major J. W. McNee, D.S.O., that, out of 500 patients questioned, only 20 per cent. had suffered from influenza in the summer. The severe cases, without exception, stated that they had had no previous attack. Allowing a margin for errors of diagnosis, these figures appear to suggest that some degree of immunity had been acquired. The following quotation from a letter received from Lieutenant-Colonel Elser, Medical Corps, U.S. Army, points in the same direction:

'In July about 80 per cent. of the Command at one of the Base hospitals (numbering approximately 228) suffered from "3-day fever", while in this present epidemic very few cases developed in this Command, and those almost invariably among individuals who had joined this organization recently.'

It cannot be claimed that these records are final or conclusive, but they point, with considerable weight, to the production of some amount of immunity. This is what might be expected, as it is difficult to believe that an epidemic can owe its cessation entirely to a diminution of virulence of the causative agent, and it is, on the whole, more simple to explain the cessation of an outbreak in terms of the production of a relative immunity in the population concerned.

III. Frequency of Haemorrhage.

Nothing is more striking than the unanimity with which all observers mention haemorrhage as a prominent feature of the disease in the autumn epidemic. This point is hardly mentioned in reports received during the summer, but records of the more severe cases show, nevertheless, that a tendency to haemorrhage existed then as later. In the post-mortem notes included in the Report by the Committee of the Director-General's Advisory Council (*B. M. J.*, November 9, 1918), it is stated that 'sub-pleural and interstitial haemorrhages were seen in 18 cases (out of 39 examined), but only as small localized areas'. Other post-mortem records of the same period afford similar information.

In reports on the autumn outbreak, on the contrary, constant reference is made to haemorrhage, blood being noted in the sputum and faeces, and epistaxis being often recorded. Notes kindly furnished by Dr. L. Stewart Sandiman, Chief Medical Superintendent, Q.M.A.A.C., show that menstruation is very frequently precipitated by attacks of influenza. The report by Captain T. H. G. Shore, R.A.M.C., lays much stress upon the constancy with which haemorrhages were noted *post mortem* and suggests their importance as a factor in the inception of the pulmonary complications of the disease. The numerous pathologists who have sent in reports are almost unanimous in noting haemorrhages in the lungs, and mention is made of bleeding in the suprarenals, the brain, the pericardium, the pleura, and other situations. Haemorrhage in the rectus muscle is frequently reported. An interesting note by Captain Murray

Lyon, R.A.M.C., records haemorrhages in the rectus muscle in 20 per cent. of 100 cases examined, and Major W. J. Wilson, R.A.M.C., describes the presence of Zenker's degeneration in the fibres of the rectus muscle in 32 out of 92 cases investigated. In 11 of these cases there was haemorrhage amongst the degenerated muscle fibres.

The haemorrhages are obviously much more common in situations, such as the lungs and rectus muscle, that are subject to strain during violent respiratory effort, as in coughing. Coughing is often continuous and trying in influenza cases, owing, perhaps, to the tracheitis so commonly found or to the irritation of enlarged and congested lymphatic glands in the neighbourhood of the trachea and bronchi. That the strain resulting from this coughing is severe is proved by the fact that emphysema is often found *post mortem* in the anterior mediastinum, and that, as in gas poisoning, air sometimes finds its way up into the tissues of the neck. It is not to be wondered at that, where toxic degeneration of muscle fibres or other tissues is so frequent, vessels often rupture under the strain of respiratory effort, or indeed without this strain where the degeneration is intense.

Stress is laid on this tendency to haemorrhage because it links up the lesions following animal inoculations with ' filtrates ' or with ' Noguchi ' cultures with those found in human cases.

A reference to the post-mortem findings in the monkeys and other animals investigated by Major H. G. Gibson, Major F. B. Bowman, and Captain J. I. Connor, shows that the production of haemorrhagic oedema is a constant occurrence. In these animals, killed at selected dates after inoculation, the lung haemorrhages form the essential pathological lesion. A striking link between the findings in infected animals and human cases is afforded by a post-mortem record of an early case in the paper by Patterson, Little, and Williams : ' In one case, whose bronchitic signs dated from only two days before death, only one small area of bright red consolidation near the hilus of the left lower lobe was found, in addition to a tremendous haemorrhagic engorgement of the whole of the lungs.'

The period of the case at which these haemorrhages occur seems to coincide with the moment of maximum ictus of the infection. It may be assumed that the causative organism cannot always or at once establish itself in complete ascendancy in the tissues of the infected person. The severity of the case will depend on whether, to what extent, and how rapidly, the virus gains the upper hand.

The tendency to haemorrhage may be taken as a rough measure of the virulence of the epidemic. Where the cases are mild, as in the summer of 1918, haemorrhages are very rare ; where severe, as in the autumn, they form the outstanding feature of the disease. It is probable that many lung haemorrhages of slight degree may be absorbed without untoward results. In monkeys, in the absence of secondary bacterial infections, fairly extensive lung haemorrhages can exist without apparent inconvenience to the animal. We know, from the study of cases of gas poisoning, how rapidly the lung can free itself from effused fluids provided that no undue strain on the respiratory mechanism leads to the inception of the vicious circle of excessive oxygen-want and consequent excessive respiratory effort. It may be assumed that many cases of influenza, clinically mild, yet

suffer from pulmonary lesions that only require a secondary bacterial infection to convert them into grave broncho-pneumonia. It appears worth while to lay great stress on this point. Even the mildest case of influenza may become grave if too early exertion or any other cause is permitted to disturb the physiological balance and thus render possible the invasion of the injured tissues by the bacteria of the respiratory tract.

IV. Morbid Anatomy and Histology.

These subjects are so thoroughly dealt with in the appended reports that very little remains to be said. An interesting suggestion by Captain A. T. Nankivell may perhaps afford an explanation of the formation of the haemorrhagic areas seen in the lungs in cases dying early in the disease and in experimental animals. Captain Nankivell thinks that these appearances may be explained 'by the occurrence of haemorrhage in some relatively distant bronchus or bronchiole, the blood flowing down, or being inspired, into healthy lung tissue, filling the majority of the alveoli there, but, owing to clotting or some mechanical factor, not penetrating into every alveolus'.

Such a haemorrhagic area of lung tissue would be liable to invasion by bacteria through the injured bronchial walls. In this connexion it is interesting to recall that practically all the secondary bacterial contaminations of influenzal lungs are organisms that grow best on media containing blood in some form.

The conclusion of Tytler, Janes, and Dobbin is that 'the interstitial broncho-pneumonia is, both from gross and microscopical examination, evidently an extension of infection through the bronchial wall to the surrounding lung tissue; not an infection of the alveoli by way of the natural passages'.

These two quotations appear to present a reasonable explanation of the early and the late phases of the lung complications in influenza.

Once started, the secondary bacterial infections may proceed throughout the injured and water-logged tissues with such unrestrained violence that the whole lung is found literally permeated by bacteria so that every alveolus is packed with them. Sections of lung from the more severe cases, appropriately stained, give a terribly clear idea of the helplessness of injured human tissues in the face of bacterial invasion.

Attention is directed to the valuable observations of Captain T. H. G. Shore, R.A.M.C., on the condition of the heart in post-mortem examinations of influenzal patients. His conclusion, that 'it would be curious if such hearts could sustain a normal intra-ventricular pressure', forms an interesting commentary on the clinical picture in these cases.

V. Bacteriology.

A. *A Filter-passer as the Primary Causative Agent.*

The question of supreme importance is whether, in view of recent observations, the causative agent of influenza is or is not a filter-

passing organism. Investigations by C. Nicolle and Lebailly and others have pointed strongly to the conclusion that the disease may be reproduced by inoculation of filtered bronchial secretions from human cases. Major H. G. Gibson, Major F. B. Bowman, and Captain J. I. Connor, working at Abbeville, have obtained results confirming and amplifying those of C. Nicolle and Lebailly in the infection of monkeys and other animals by these filtrates. This investigation, published in the *British Medical Journal* of December 14, 1918, formed, it is believed, the first British contribution to the problem of a filtrable virus in Influenza. Further, applying the Noguchi technique in the manner employed by G. Foster in the case of common colds, they have been able to obtain a growth of very minute anaerobic Gram-positive globoid bodies, both from filtrates and from organs of infected animals, and, on injecting these cultures into monkeys, rabbits, and guinea-pigs, have reproduced the haemorrhagic pulmonary lesions now coming to be regarded as characteristic of the disease in animals.

The cultural work of these observers affords independent confirmation to that carried out a few months earlier by Captain J. A. Wilson at Etaples (Bradford, Bashford, and Wilson, *B. M. J.*, February 1, 1919). Major Gibson, Major Bowman, and Captain Connor were unaware of Captain Wilson's work which, owing to its forming part of a larger research, was not at once made public, and their cultures were obtained with a modification of the Noguchi technique differing in certain particulars from that employed at Etaples.

That two series of observations, carried out independently, should thus confirm each other, greatly strengthens the case for the new organism. It is necessary, however, to preserve a critical attitude until the question has been more thoroughly investigated. ' Passage work ' has been, so far, practically limited to animals owing to the danger of using human volunteers and to the fallacies incident to human ' passage ' experiments during a pandemic. It is not always safe to regard the results of animal experiments as applicable to man. There are many difficulties to be overcome in working with so-called ' filter-passing ' organisms, or indeed with any organisms of very minute size, and these difficulties and limitations must be taken into account in considering the work now brought forward.

An obvious criticism is that whereas the incubation period of influenza is known to be definitely in the vicinity of forty-eight hours, the animals infected by Nicolle and Lebailly and by Gibson, Bowman, and Connor did not show signs of illness before the sixth or seventh day. This objection can be parried theoretically in several ways, but it must be admitted that controversy on the point is still in the realm of theory and that further knowledge can only be gained by continued experimental work.

All that can now be stated is that a very good case has been made out for a ' filter-passer ' as the causative agent in influenza, and that, if the haemorrhagic lung oedema produced by inoculation of animals be accepted as identical with human influenza, Koch's postulates can be said to have been fulfilled.

B. *Other Organisms as Secondary Infective Agents.*

(a) *B. influenzae (Pfeiffer).* Had it not been for these recent observations which have gone so far towards demonstrating the etiological rôle of a filter-passing organism, the evidence in favour of *B. influenzae* would probably have been regarded as conclusive. As it is, this bacillus must still be admitted to be the earliest and most constant 'associate', appearing on the scene within the first hours of the attack. To what extent it facilitates the operations of other and more virulent germs still remains to be discovered.

Tytler, Janes, and Dobbin, in commenting on their finding of *B. influenzae* in 90 per cent. of the cases examined, sum up as follows: 'Allowing for errors of technique, unavoidable in a series of this size . . . this means that the bacillus was constantly present.'

The presence of *B. influenzae* in a high proportion of cases within the first week of the disease, and at a time when streptococci and pneumococci were much less frequently found, is well shown in the chart in the paper by Patterson, Little, and Williams. These observers cultivated *B. influenzae* from sixty-two out of sixty-three fatal cases.

Not only is *B. influenzae* present in nearly every case but it tends to evoke immune responses early in the disease.

In confirmation of the work of Captain J. Cruickshank in the summer outbreak, the presence of specific agglutinins for this organism has been demonstrated, during the autumn epidemic, by Captain A. Fleming, Captain H. H. Perry, Major H. G. Gibson, Major F. B. Bowman, and by Captain P. Hartley whose report on this subject is included in Part II; but the reaction has not yet been shown to be either so constant or so clear-cut as to serve as a reliable diagnostic method. Its interest lies, rather, in the evidence which it affords of the early presence of *B. Pfeiffer* as an organism actually evoking immunizing responses in the cases examined. It is worthy of remark that the agglutination was most complete and most constant in blood-samples collected up to and including the seventh day, after which the phenomenon was less frequently demonstrated.

B. influenzae has been cultivated from the blood stream on several occasions, though this is rare during the normal course of the disease. That it invades the circulation fairly frequently, as a terminal infection, is shown by the observations of Lieutenant B. A. I. Peters, R.A.M.C., who recovered it from the heart's blood in nine out of forty-one cases examined by him *post mortem.*

At the same time nothing is more curious, in view of the constant presence of *B. Pfeiffer* in influenza cases in France and elsewhere, than the negative reports by workers of undoubted merit in certain other localities. In the *Medical Supplement of the Daily Review of the Foreign Press* of September 1, 1918, negative or inconclusive findings of the *B. influenzae* are recorded by Gruber, Friedmann, Uhlenhuth, Kolle, and even Pfeiffer himself.

The technique for the isolation of *B. influenzae* has been much improved of recent months, and it becomes a question of interest as to how frequently this organism will be found present in the sputum of chest cases other than influenza during times when this

disease is not epidemic. Figures kindly given me by Colonel J. F. Siler, Medical Corps, U.S. Army, Director of Laboratories, American Expeditionary Force, show that out of 2,179 healthy persons serving in medical units, 595, or nearly 27 per cent., were found to be healthy ' carriers ' of *B. influenzae* at a time when the disease was prevalent ; a percentage not very much below that found in early cases of influenza examined by throat-swabbing before sputum is available. It is possible that *B. influenzae* may be much more common than was supposed in the normal respiratory tract.

(b) *Other Organisms.* The papers included in this report give a clear picture of the general prevalence of other organisms as secondary infective agents in influenza in France. Streptococci, pneumococci, staphylococci, and meningococci are the more important organisms found. These, with the exception of the pneumococci, seldom give rise to septicaemic infections except in fatal cases shortly before death ; but the conditions prevailing in influenzal lungs greatly favour their activity, and the principal danger to influenzal cases appears to be the tendency to secondary infections of the damaged pulmonary tissues.

It would seem that the secondary bacteriological findings conform to the varying flora of the human respiratory tract in different localities, the contradictory nature of some of the reports depending upon this factor ; thus haemolytic streptococci may be found as the commonest organisms in one place while they are replaced almost entirely by *Streptococcus viridans* in another. Much confusion has undoubtedly arisen in differentiating pneumococci from some of the capsulated and lanceolate streptococci, but there seems no doubt that the proportion of positive findings of true pneumococci varies greatly in different localities.

The paper by Captains A. V. Bock and J. L. Stoddard of the Medical Corps, U.S. Army, recording their findings in a series of cases of which 64 per cent. were Americans, will be read with interest in this connexion and should be compared with the findings of Tytler, Janes, and Dobbin, at No. 3 Canadian General Hospital, where lobar pneumonia was hardly ever observed.

The tendency for the pneumococcus to gain entrance to the blood stream is well brought out in this paper and is confirmed by the isolation of this organism in blood cultures in twelve out of forty-four cases by Dr. Little and Miss Williams.

Major Tytler calls attention to the very high percentage of staphylococcal infections, and this is confirmed by a report by Major W. J. Wilson on cases examined in the same area. Similar findings are reported from Malta by Captain Adam Patrick (*The Lancet*, January 25, 1919). On the other hand, staphylococci do not take a prominent place amongst the organisms isolated from the cases, mostly German prisoners of war, investigated by the Abbeville Research Team.

With all these variations in the bacteria of secondary infections, the morbid anatomy and morbid histology remain wonderfully constant, the differences representing stages in a progressive process rather than true variations of type. Except for the fact that the rare cases of lobar pneumonia are usually found to be associated with true pneumococci, the variations in morbid anatomy appear

but little connected with variations in the associated bacteria. To quote from the paper by Tytler, Janes, and Dobbin :

' An analysis of the relation of the type of organism isolated to the type of lesion present does not reveal any features striking enough to definitely ascribe any type of lesion to infection with any one particular organism.'

An important factor underlying the production of secondary lung complications would appear to be the degree of injury to pulmonary tissues by the primary etiological agent of the disease. The resulting areas of haemorrhagic oedema afford a ready portal of entry and ideal conditions of growth for the organisms already existing upon the surface of the respiratory tract.

The whole clinical and pathological picture becomes intelligible if we can postulate a primary etiological agent acting locally upon the respiratory surfaces and generally through its toxic products in such a manner as to prepare the way for invasion by the prevailing respiratory flora. The work of Gibson, Bowman, and Connor at Abbeville and of Wilson at Etaples holds out a promise that this explanatory link may now be supplied.

PART I

I. THE ETIOLOGY OF INFLUENZA: A FILTRABLE VIRUS AS THE CAUSE

WITH SOME NOTES ON THE CULTURE OF THE VIRUS BY THE METHOD OF NOGUCHI

BY

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SINCE the results of our preliminary experiments were reported in the *British Medical Journal* of December 14, 1918,[1] in an article entitled ' A Filtrable Virus as the Cause of the Early Stage of the Present Epidemic of Influenza ', further work has been carried out which confirms that already reported.

We have also attempted to cultivate the ' filtrable virus ' of influenza and have made experiments in the transmission of the disease to animals by inoculation with the cultures so obtained.

These cultural experiments were suggested by one of us (H. G. G.) at an early stage of the work, and later, a paper by G. Foster,[2] to which we had access, clearly showed that this part of the work might prove to be a very important part of the investigation.

The animals used by us for experimental purposes have been baboons, *Macacus rhesus* monkeys, rabbits, guinea-pigs, and mice. By the inoculation of sputum, both filtered and unfiltered, from cases of influenza, we have succeeded in producing, in all these animals, lesions that appear similar to those seen in human cases of the disease.

For experiments with blood transmission we used *M. rhesus* monkeys and mice. Although the results obtained in the only two cases in which monkeys were inoculated with blood were not wholly satisfactory, the experiments with mice gave a high percentage of positive results and it seems to be possible to transmit the virus by means of infected blood to the latter animals. It should be noted that the mice received a very much larger dose per body weight than the monkeys and this factor, no doubt, played a part in the results obtained.

With cultures it was found to be possible to infect baboons, monkeys, rabbits, guinea-pigs, and mice. Owing to the grave nature of the influenza epidemic in progress, it was not thought advisable to attempt any experiments in the transmission of the disease to human volunteers, both on account of the danger involved and owing to the fact that there would have been no guarantee, in the face of so

widely-spread an epidemic, that the individual to be experimented upon had not already been exposed to infection.

Before going on to report the results obtained it seems desirable to describe, in some detail, the technique employed by us in our investigations.

I. Housing of Animals.[1]

A. *Monkeys.*

The monkeys were kept for at least a week after their arrival before being used for any experimental purpose, in order that they might become accustomed to their new surroundings and that we might be sure that they were in good health. The advisability of this course was borne out by the fact that two of our monkeys developed other diseases while under observation, one dying two days after arrival from pneumonia associated with M'Gowan's bacillus of distemper and to which reference is made later, and the other from tuberculosis.

The monkeys, from the time that they arrived, were kept in separate cages. These cages were maintained in clean condition by washing out with cresol solution. The cages were separated from each other by means of wooden partitions and the whole house in which the monkeys was kept were warmed by means of a stove.

B. *Rabbits, Guinea-pigs, and Mice.*

Until used for experimental purposes, all the rabbits were kept together and the same applies to the guinea-pigs and mice. After an animal had been inoculated it was kept in a cage by itself and rigorously segregated from the remainder.

No cage used for the purpose of housing any animal, whether inoculated or not, was used for fresh animals until it had been thoroughly disinfected.

II. Preparation of Material used for Inoculation.

A. *Sputum.*

On the sputum being brought to the laboratory in clean sputum pots, a portion was examined by means of a direct smear and by culture. The cultures were made on defibrinated human blood agar and in glucose serum broth. From various samples of the sputum used, pneumococci, streptococci of the haemolytic and *viridans* groups, *M. catarrhalis*, staphylococci and *B. influenzae* (Pfeiffer) were isolated.

The remainder of the sputum was then taken and ten times its volume of normal saline was added to it. This was placed in a sterile milk bottle with a clamp stopper, which contained sterile glass beads, and it was then vigorously shaken by hand for five minutes. The contents of the bottle were then centrifuged for one minute and a half at from 1,500 to 2,000 revolutions per minute, after which the supernatant fluid was decanted into a sterile flask.

At this stage the mixture was either used for inoculation in its unfiltered state, in which case a culture was first taken from it,

or was filtered through a Chamberland L. 1 bis or Chamberland F. filter under a negative pressure of 50 cm. of mercury. The filtrate was then tested for sterility, using the same media as mentioned above for culturing the sputum. In no case was any growth of non-filtrable organisms obtained. The filtrate obtained was used for inoculation, except on two occasions, when a portion of the filtrate was first heated in a water bath at 71° C. for half an hour and was then inoculated subcutaneously into mice.

B. *Blood.*

In the case of blood the technique employed was slightly different, The blood was drawn off from the median basilic vein by a syringe with the usual aseptic precautions.

In the majority of cases the animals to be inoculated were taken into the ward in which the patient suffering from influenza was in bed. Usually about 10 c.c. of blood were drawn off, a few drops were sown into a defibrinated-blood agar plate and 3 c.c. inoculated into mice which were the animals chiefly used for these experiments.

In one instance, where an attempt to infect monkeys was to be made with unfiltered and filtered blood, 20 c.c. were drawn off by means of a needle and sterile rubber tubing which led into a flask containing 2 c.c. of 10 per cent. sodium citrate. Cultures were taken from this and proved sterile. The remainder was divided into two parts, A and B.

Of these two parts, 10 c.c. of A were placed in a sterile measuring cylinder and 40 c.c. of sterile distilled water were added to lake the blood. This was cultured and proved sterile for non-filtrable organisms. The laked blood was then filtered through a Chamberland L. 1 bis filter and the filtrate similarly tested and proved free from non-filtrable germs. This was again divided into two parts, one of which was left unheated and the other heated to 55° C. for half an hour in a water bath.

In the meanwhile, the part B mentioned above and which consisted of 10 c.c. had been left standing at room temperature during the process of preparing part A, in order that the whole blood might have been kept for a similar period outside the body before inoculating it into monkeys. This period was one of about two and a half hours.

III. A General Picture of the Clinical and Pathological Results Obtained in Animals Inoculated with the Virus in those Instances in which a Positive Finding was Recorded.

The clinical and pathological picture obtained was the same, whether unfiltered or filtered influenzal sputum was used as the infecting agent.

A. *Clinical.*

This varied somewhat with the kind of animal employed. In monkeys a clinical picture of depression, anaemia, with a staring coat and in one instance diarrhoea was obtained, though this was by no means constant, and we were struck with the degree of damage

that might be present in the lungs without any marked clinical symptoms or signs that anything was amiss with the animal. *When symptoms were noticed, they appeared on the fifth to seventh day after inoculation.*

The temperature charts of the monkeys gave us very little clue as to what one might expect to find *post mortem* after the animal was killed. The temperatures of the monkeys were taken twice daily from the time we received them. The average temperature seemed to be about 102° F., but the range of temperature obtained with normal monkeys was never noteworthy, temperatures of anything between 99·6° F.–104° F. being recorded. After inoculation there was never any marked rise in temperature, but we gained a general impression that the temperatures were inclined to swing more after this had taken place. The other animals, except mice, showed no marked symptoms.

One or two of the mice inoculated with the virus showed some sign of respiratory distress at varying intervals up to three days after the inoculation, but these subsequently recovered. Some of the mice died when a large dose was given and in only two cases was the death of the animals witnessed. In these two cases the mice were seen lying on their backs with obvious respiratory distress and clutching at the cork in their cage with their front legs. In all other cases of death in the experimental animals, they were found dead in their cages. In one instance two mice, previously infected with coccidiosis, died, while another mouse which was inoculated with the same filtrate and which had no other intercurrent affection showed no symptoms, although when killed its lungs presented the usual picture. In another case five out of six mice inoculated with large doses of blood from a case of influenza died within forty-eight hours. As a rule, we were struck by the paucity of the symptoms in animals inoculated with unfiltered or filtered sputum given in those cases which presented gross pathological lesions in the lungs after being killed.

B. *Pathological.*

The pathological lesions found in all animals in which positive results were obtained, whether they died or were killed, closely resembled each other and also the conditions found in man in cases which died at an early stage in the disease. The condition found was the same in the monkey, rabbit, guinea-pig, and mouse. For the purpose of this communication it is proposed to give a general description of the macroscopic and microscopic lesions observed.

(a) *Macroscopic appearances. Respiratory tract.* The larynx of the animal was generally normal, but in one or two instances there was some slight injection present.

The trachea was often red and congested, though this condition was not absolutely constant. A much more constant feature was the presence of frothy blood-stained fluid in the lower part of the trachea which oozed up from the bronchi. *The lungs* themselves in every case showed patches of a haemorrhagic nature of varying sizes. For the most part they were more marked on the posterior than on the anterior surfaces of the lungs.

These haemorrhagic areas were not only seen sub-pleurally, in which case they showed a tendency to spread into the lung substance to a slight extent, but, on section, they were also seen to be scattered through the lung substance itself, varying greatly in size, and in one or two cases the lung substance was of a dark red to purple colour and presented a wet appearance. In nearly all cases the lower lobes were the ones most affected. On cutting into the lung substance in several instances a viscid dark purple fluid dripped from the cut surface, closely resembling that seen in human cases of influenza. No evidence of broncho-pneumonia was seen in any animal lung examined by us.

Bronchial glands were found to be enlarged in some instances.

The liver was not markedly affected in the majority of the cases, though in a few there was a certain suggestion of yellowish mottling.

The other organs were apparently normal to the naked eye. The most marked changes were always found in the respiratory tract.

Pleural sacs. In the large majority of cases there was no evidence of any pleurisy, but in the case of one monkey, which was killed about three weeks after its inoculation with filtered sputum from a case of influenza, there were some adhesions present, apparently of between two and three weeks' standing. In the case of a monkey inoculated with unfiltered sputum there were some recent adhesions between the lower lobe of the lung and the diaphragm and between the lobes of the lung. In one mouse also the lungs on both sides were bound down to the parietal pleurae.

(b) *Microscopic appearances. Lungs.* Examination of over 200 sections. (See Plates VIII, IX, X.)

Microscopic examination of the lungs of affected animals presented a very similar picture to certain areas seen by us in some of the human lungs taken *post mortem* from men who had died at an early stage of the disease. Judging from the examination of mice dying at a very early stage, the first miscrocopic sign seems to be an acute dilatation of the capillaries in the alveolar walls, and some sections showed a certain amount of actual hāemorrhage into the alveoli. In the most marked animal lungs there was an almost universal consolidation, the alveoli being filled with what appeared to be an inflammatory and haemorrhagic exudate.

The bronchi and larger vessels were often seen filled with the same exudate, and the impression was conveyed that the circulation in some areas had been stopped. The mucous membrane of the bronchi was usually intact and some cells showed a mucoid accumulation. Leucocytic accumulation was, as a rule, scanty, but some of the lungs taken from animals killed at a later date showed a certain amount of leucocytic infiltration, and in one monkey killed three weeks after inoculation there was some destruction of the lining membrance of the smaller bronchi.

In no instance was any approach to grey hepatization seen. The high power in some cases showed the exudate in the alveoli to consist of an amorphous material and in several instances the remnants of degenerate red-blood corpuscles were seen, some of which still maintained their original shape.

IV. Animal Inoculation and Cultural Experiments with Filtrable Virus.

The work may be divided into four parts :

A. Inoculation of animals with sputum from cases of influenza.
B. Inoculation of animals with blood from cases of influenza.
C. Passage of the virus from animal to animal.
D. Cultural experiments and inoculation of cultures into animals.

A. *Inoculation of animals with Sputum from Cases of Influenza.*

(a) *Source of infected material.* The sputum used was as a rule collected as early as possible in the disease. As uncomplicated cases of influenza as a rule present a pyrexial period of only a few days' duration we considered that it would be during those few days that we should have the greatest chance of recovering the virus. As a rule a certain amount of sputum was obtainable on the second or third day, and this, generally speaking, was frothy, of a greyish yellow colour, tenacious, often markedly bloodstained, and did not present the numular appearance seen in cases of bronchitis.

(b) *Method of inoculation and size of dose given. Sputum.* Emulsions of unfiltered and filtered sputum were inoculated by the same routes and the size of the dose was the same in each case. When dealing with monkeys the methods of administration of unfiltered sputum reported by Nicolle and Lebailly was followed.

This consisted in injecting 0·25 c.c. of the emulsion under the conjunctiva of each eye and instilling 0·5 c.c. of the emulsion up the animal's nose. In one instance we inoculated one monkey by the conjunctival route alone, and gave another monkey 0·5 c.c. of the same filtrate up its nostrils alone. The former gave a positive result while the latter proved negative.

Rabbits. These animals were given 1·2 c.c. of the filtered sputum either intravenously or subcutaneously. Positive results were obtained by both methods.

Guinea-pigs and Mice were always inoculated subcutaneously whether unfiltered or filtered sputum was used. Guinea-pigs were usually given 1 c.c. and mice, when used, received 0·25 c.c. of the unfiltered or filtered sputum.

In dealing with the results obtained it is convenient to separate the various animals employed into different groups, taking the monkeys first, we may divide them into two main groups according to whether the sputum used as the infecting agent was taken from the patient during the first three days of the disease or at a later date. The results are shown in the subjoined table :

Group I. Sputum collected early.

Type of Monkey.	*Material used for inoculation.*	*Date of disease on which sputum was taken.*	*Result.*
1. *M. rhesus*	Unfiltered sputum	3rd day	Positive
2. *M. rhesus*	,, ,,	3rd day	Doubtful
*3. *M. rhesus*	Filtered ,,	3rd day	Positive
*4. *M. rhesus*	,, ,,	3rd day	,,
*5. *M. rhesus*	,, ,,	2/3 day	,,
*6. *M. rhesus*	,, ,,	3rd day	,,
7. *M. rhesus*	,, ,,	3rd day	Negative

* See Plates III and IV, IX and X.

This gives 80·0 per cent. positive results among the *M. rhesus* monkeys inoculated with *filtered* sputum taken from cases of influenza before the end of the third day of the disease. Out of the two monkeys inoculated with *unfiltered* sputum taken during the same period one positive result was obtained and in the other instance although the macroscopic examination revealed little, the microscope showed patches of acute inflammation with the typical inflammatory exudate. Thus 85·7 per cent. of all monkeys inoculated with sputum filtered or otherwise gave positive results if the doubtful case is included; if excluded 71·4 per cent. were positive.

Where sputum taken at a later date was inoculated the results obtained were less satisfactory. One monkey was inoculated with *unfiltered* sputum taken on the sixth day from a case of influenza. A negative result was obtained. A monkey inoculated with the filtered sputum from the same case also showed no signs of disease *post mortem*. A monkey inoculated with filtered sputum from a case of influenza taken on the fifth day gave a positive result.

Group II. Sputum collected later.

Type of Monkey.	*Material used for inoculation.*	*Date of disease on which sputum was taken.*	*Result.*
1. *M. rhesus*	Unfiltered sputum	6th day	Negative
*2 *M. rhesus*	Filtered ,,	6th day	,,
3 *M. rhesus*	,, ,,	5th day	Positive

* See Plate VII A.

In this instance two out of three attempts failed, or only 33·3 per cent. were positive. These figures are very small and insufficient to justify any final opinion as to the period in which it may be impossible to obtain the virus from a case of influenza, but with the number of monkeys which we had it was thought more profitable to concentrate our attention on sputum obtained at an earlier date. The time up to which the virus can be recovered must form the basis for further work on this subject.

An attempt to infect a monkey with filtered sputum instilled up its nostrils without any subconjunctival injection failed, although subconjunctival injection, without nasal instillation, of the same filtrate, gave a positive result in another monkey.

Controls. Two monkeys were inoculated with filtered sputum obtained from two cases of acute bronchitis on the third day of the disease. The sputum was prepared and filtered in the same way and the monkeys were inoculated subconjunctivally and by nasal instillation in each case. They were given the same dose, but were both negative as regards symptoms and both macroscopic and microscopic examination of their lungs.

Another monkey died two days after arrival with a lobar pneumonia associated with M'Gowan's bacillus of Distemper. The post-mortem findings were totally different from those seen in our experimental animals. Microscopically there was no great engorgement of capillaries and no haemorrhagic exudate in the alveoli.

The obtaining or not of a positive result by means of the injection of monkeys with filtrable viruses depends very much more on the

dose given than in the case of experimental infections with non-filtrable germs. At present it is not possible to determine the dose that is being given. It is known that while these viruses, as a rule, reproduce the picture of the human disease, as, for instance, in the case of polyomyelitis, the certainty of producing a positive result is not nearly so great as when dealing with the non-filtrable bacteria which are pathogenic for animals. In view of these facts the animal experiments with monkeys appear to us to be quite significant as far as they go.

Rabbits. Four rabbits were inoculated. Of these, two were inoculated with filtered sputum taken on the third day from a case of influenza. One of these rabbits was given 1 c.c. of the filtrate intravenously and the other 1 c.c. of the filtrate subcutaneously. Both inoculations were followed by a positive result. One lung, taken from the rabbit that had been inoculated subcutaneously, was ground up in a mortar with normal saline and the extract filtered. The filtrate was inoculated into a second rabbit (see Passage Experiments). The kidney of the rabbit receiving the intravenous dose was placed in a Noguchi tube with ascites fluid (see Cultural Experiments).

One rabbit was inoculated subcutaneously with 2 c.c. of a filtrate of bronchial pus taken, *post mortem*, from a man who died from influenza on the twelfth day of the disease. The result was completely negative and tends to confirm our opinion that it is likely that the virus is not recoverable after the first few days of the disease.

Control. A rabbit was given 2 c.c. of a filtered sputum taken on the third day of the disease in a case of acute bronchitis. Result negative.

Guinea-Pigs. Four guinea-pigs were inoculated. Two of these were inoculated with sputum taken on the night of the second day and morning of the third day from a case of influenza. One guinea-pig was given 1 c.c. of the diluted (1–11) but unfiltered sputum, and the other received 1 c.c. of the same dilution of this sputum, filtered. In each case a positive result was obtained.

A guinea-pig inoculated subcutaneously with 1 c.c. of the filtered bronchial pus mentioned above gave a negative result.

Control. A guinea-pig inoculated subcutaneously with 1 c.c. of the filtrate of sputum from a case of acute bronchitis also gave a negative result.

Mice. Altogether, fourteen mice were inoculated with sputum, unfiltered or filtered, from various sources.

These may be divided into those inoculated with :

(*a*) Unfiltered sputum from cases of influenza.

(*b*) Filtered sputum taken during the first three days of the disease in cases of influenza.

(*c*) Heated filtered sputum taken during the first three days of the disease in cases of influenza. (Heated to 71° for half an hour.)

(*d*) Filtered sputum taken on the sixth day of the disease and later from influenza cases.

(*e*) Filtered sputum taken on the third day of the disease from a case of acute bronchitis.

Under Group (*a*) 4 mice were inoculated :
1 gave a negative result.
2 died of pneumococcal infection.
1 died of streptococcal infection.
Under Group (*b*) 5 mice were inoculated :
4 positive results.
1 was killed 24 hours after inoculation—result negative.
Under Group (*c*) 2 mice were inoculated :
2 negative results. (Mice inoculated with the same filtrate unheated all died.)
Under Group (*d*) 2 mice were inoculated :
2 negative results.
Under Group (*e*) *Control,* 1 mouse was inoculated :
1 negative result.

B. *Inoculation of Animals with Blood from Cases of Influenza.*

Experiment I. For this experiment 24 c.c. of blood were drawn off aseptically from the median basilic vein of a man who had been forty-eight hours ill with influenza. At the time the blood was taken his temperature had begun to fall, and this reached normal on the third day, after which he made an uninterrupted recovery. The blood was run off directly from the vein into a flask containing 2 c.c. of 10 per cent. sodium citrate. The blood was then divided into two parts. One part was left at room temperature, and 10 c.c. of the other was diluted with 40 c.c. of distilled water to lake the blood.

Cultures were made in the usual way, both from the whole and laked blood, and proved sterile in each case after a week's incubation. The laked blood was then passed through a Chamberland L. 1 bis filter under a negative pressure of 50 c.c. of mercury. The filtrate also proved sterile with regard to non-filtrable organisms.

A *M. rhesus* monkey (No. 8) was inoculated subcutaneously with 5 c.c. of the whole blood which had been standing at laboratory temperature for six hours. This monkey showed no symptoms up to the seventh day, after which he was killed. *Post mortem* nothing abnormal was seen, nor was any abnormality observed in its lungs on microscopical examination.

A *M. rhesus* monkey (No. 6) was inoculated subconjunctively in each eye with 0·25 c.c. of the filtrate and 0·5 c.c. was instilled up its nostrils. Five days later it was 'moping' somewhat and its temperature that afternoon rose to 108° C. The condition remained the same on the following day, and on the seventh day it was killed. The post-mortem findings were as follows :

The monkey was thin and did not appear to be well nourished.

Larynx. This was somewhat injected.

Trachea. The mucous membrane was markedly red between the tracheal rings.

Lungs. Beyond retaining their shape when placed on the table, the lungs of this animal presented no abnormal features either externally or on section when examined macroscopically.

Other organs. These were normal.

Microscopic appearances. The section of the lung showed some patchy inflammatory changes in which small groups of alveoli had become filled with inflammatory exudate, and this was already invaded by leucocytes. These areas showed some engorgement of capillaries. The leucocytic accumulation was more marked round the smaller bronchi, and some desquamation of the mucous membrane was present.

Bacteriology. Bacteriological examination of the organs of both these animals gave negative results in culture.

In addition to the above inoculations, one mouse received 0·25 c.c. of the whole blood subcutaneously (Mouse W. 1).

One mouse received 0·25 c.c. of the filtered blood subcutaneously (Mouse W. 2) and one mouse received 0·25 c.c. of the filtered blood which had been heated to 55° C. for half an hour (Mouse W. 3). Unfortunately two of the mice employed (Mice W. 1 and W. 3) were already infected with coccidiosis. The mouse receiving the unfiltered blood died during the night following on the injection. *Post mortem* the lungs appeared very haemorrhagic and small white areas were present on both lungs. Under the microscope the lung presented an acute haemorrhagic condition superimposed on coccidiosis.

The mouse receiving the filtered blood (untreated) was *killed* on the fourth day after the inoculation. *Post mortem* the lungs showed red haemorrhage-like patches on both lungs, especially on the posterior surfaces. No infection by coccidia was found in this animal.

Microscopically, the lungs were seen to be in a condition of congestion with acute inflammation with the usual exudate (see Plate IX B, Mouse W. 2). The mouse (Mouse 3) which received the heated filtrate died on the third day after inoculation. This mouse was also infected with coccidia. The lungs *post mortem* were apparently haemorrhagic and discrete white areas were present over their surfaces. Microscopically there was seen to be present a condition of acute inflammation in addition to an infection with coccidia. The bacterial investigation of the blood and lungs of these mice gave constant negative results. The last experiment points to the probability that the filtrable virus resists heating to a temperature of 55° C. for half an hour.

Experiment II. Three other mice were inoculated subcutaneously with whole blood which was taken aseptically from the median basilic veins of patients suffering from influenza. In one instance the blood was removed on the first day of the disease and in the other two on the second day. The mice in all instances were inoculated with 1 c.c. of blood subcutaneously. The inoculations were carried out in the ward in which the patient was immediately the blood was withdrawn from the vein. The mouse inoculated with the blood taken on the first day of the disease died eight days later. *Post mortem* the lungs showed definite haemorrhage-like areas over both lungs. Microscopically, the alveoli were filled with an inflammatory exudate, and there was also present congestion of the capillaries. One mouse inoculated with blood taken on the second day of the disease was killed eight days later.

Post-mortem findings. On opening the thorax the lungs were found bound down and extremely difficult to remove. They showed deep

red staining in places and were not of the usual pink colour. This condition was present on all the lobes of the lungs.

Microscopically marked inflammatory reaction was seen to be present. There was some haemorrhagic exudate scattered through the lung substance in the alveoli. The section also showed thrombosis of the vessels and marked small cell accumulation round some of the bronchi.

Another mouse, inoculated with blood taken on the second day of the disease and killed on the sixth day, showed the same haemorrhage-like patches over the lungs *post mortem*. Microscopically, there was again present acute congestion with haemorrhage into the alveoli, acute inflammation, and some leucocytic reaction.

Bacteriology. This examination gave negative results in each case.

As Controls to these three mice, two mice were inoculated with, in one case, 1·25 c.c. of normal human blood, and in the other, 1 c.c. of normal human blood. In neither instance was an abnormality found *post mortem* either macroscopically or microscopically.

Experiment III. This experiment was undertaken to attempt to obtain information as to the earliest time after inoculation into mice of blood from influenza cases that one may expect to find any pathological lesions in the lungs of those animals. Six mice were inoculated, each with 1 c.c. of blood taken from a case of influenza during the first twenty-four hours of the disease. These mice were each given 1 c.c. of blood subcutaneously, and it was intended to kill these animals at varying intervals up to eight days after inoculation. However, this intention was frustrated by the fact that five out of the six mice died during the first forty-eight hours after they received the injection.

The results are best shown in tabular form :

No. of Mouse.	*Inoculated.*	*Died.*	*Appearance of lung Post-M.*
U. 1	2.12.18	Same night	Lungs showed nothing abnormal (inoculated intraperitoneally).
U. 2	,,	Next morning	Both lungs showed haemorrhagic-like patches.
U. 3	,,	Same night	Nothing abnormal seen in lungs (inoculated intraperitoneally).
U. 4	,,	Next morning	L. lung showed haemorrhagic-like patches.
U. 5	,,	Night of 3.12.18	Haemorrhagic-like patches on both lower lobes.
U. 6	,,	Killed 3.12.18	Lungs apparently normal.

Of the six lungs examined microscopically, two showed acute inflammatory reaction with congestion in one instance. Of the remainder, three showed acute congestion only and one some leucocytic infiltration.

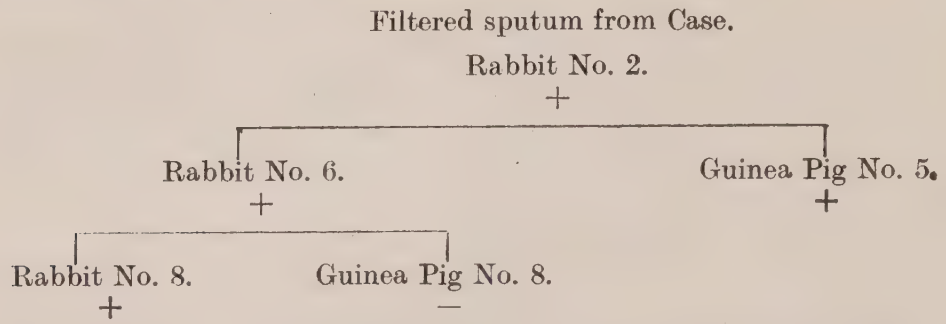
C. *Passage of the Virus from Animal to Animal.*

Filtered sputum from an early human case of influenza was administered to a group of two monkeys (Nos. 11 and 7), two rabbits (Nos. 1 and 2), and two mice (Nos. Z 1 and Z 2) on December 9, 1918. Of this group, Monkey 7 received no injection but the filtered sputum was merely instilled into the nose. This animal remained

negative. Mouse Z 2 was given an injection of *heated* filtrate and, as was to be expected, remained negative.

All the other animals were inoculated with the unheated filtrate and all gave positive results.

Of these animals rabbit 2 was selected to provide material for passage experiments.



Rabbit No. 2. The history of this rabbit was as follows :

9. 12. 18. *Subcutaneous inoculation of* 1 *c.c. of filtrate from sputum.* No symptoms of illness noted after inoculation.

15. 12. 18. Killed and examined *post mortem.*

Findings. The lower lobes of both lungs showed purplish discoloration and haemorrhagic areas. Microscopically, irregular acute inflammatory changes, with early leucocytic reaction, were noted in sections of the lungs, together with engorgement of the capillaries.

Bacteriological examination of the lungs was negative so far as concerns non-filterable organisms.

The lower lobe of the left lung of this rabbit was now removed, crushed, extracted with normal saline, and the extract passed through a Chamberland F. Filter and used as the inoculum for further passage experiments, being injected into rabbit No. 6 and guinea-pig No. 5.

Rabbit No. 6.

15. 12. 18. *Subcutaneous inoculation of* 2 *c.c. of filtered lung extract from rabbit No.* 2.

23. 12. 18. Killed and examined *post mortem.*

Findings.

Trachea. There is found some tracheitis in the lower part of the trachea which is filled with a frothy blood-stained fluid which oozes up from the bronchi.

Pleura. Some fluid present in each pleural sac.

Lungs. Retain shape on being stood on the table. The anterior surface of the right lung shows a patch of haemorrhage at the root of the upper lobe. The anterior surface of the left lung presents very little abnormality. The posterior surface of both lungs is uniformly of a dark red colour. On section, the lung substance is seen to be reddish-brown in patches and some frothy blood-stained fluid drips from the cut surface. Cultures on blood-agar and glucose broth were found to be sterile so far as regards non-filtrable germs. Lung substance from this animal was emulsified and inoculated into rabbit No. 8 and guinea-pig No. 8.

Guinea-Pig No. 5.

15. 12. 18. *Subcutaneous inoculation of* 2 *c.c. of filtered lung extract from rabbit No.* 2.

23. 12. 18. Killed and examined *post mortem.*

Both lungs were found to show large dark-red patches over both anterior and posterior surfaces.

Rabbit No. 8.

23. 12. 18. *Intravenous inoculation of* 2 *c.c. of lung extract from rabbit No.* 6.

Died about one hour after inoculation.

24. 12. 18. *Post-mortem examination. Peritoneal cavity* clear and glistening. Bowel generally pale and yellowish.

Trachea. The upper part shows the blood-vessels between rings outlined in deep red, and an extreme degree of congestion seems to be present between all the cartilaginous rings. Frothy fluid wells up in the trachea from the bronchi.

Pleura. No evidence of pleuritis on either side. No fluid present.

Lungs. Both lungs appear much the same. Generally they are pink with an overlying crimson and deep red coloration which looks haemorrhagic. It seems, in the gross, to be an extreme grade of what has previously been seen in infected rabbits' lungs.

On section, the surface drips bloody fluid and shows numerous deep red small haemorrhagic areas on the cut surface.

Thymus. Enlarged. Soft. Vessels deep red and engorged. Large numbers of deep purple haemorrhages seen on surface.

Kidneys. Deep red. Drip blood on section. Capsule strips easily.

Liver. Deep red. On section, blood drips from surface and lobules appear outlined in deep red.

Guinea-Pig No. 8.

23. 12. 18. *Subcutaneous inoculation of* 2 *c.c. of lung extract from rabbit No.* 6.

31. 12. 18. Killed and examined *post mortem.*

The lungs and other organs appear to be normal.

Remarks. In the above series of experiments a tendency to increase of virulence was noted in passage through rabbits. The lesions found in rabbit No. 2 were of only moderate severity. Those produced in rabbit No. 6 by inoculation of material from rabbit No. 2 were much more severe. The inoculation of lung extract from rabbit No. 6 into rabbit No. 8 led to the rapid death of the latter, the symptoms and post-mortem appearances being very striking and suggesting that the inoculum may have contained a toxin of high virulence in addition to the living virus. It should be noted that this animal received an intravenous inoculation, so that any toxin introduced would have been rapidly distributed throughout the organism. It is a remarkable thing, however, that an equivalent amount of the same lung extract proved harmless for guinea-pig No. 8, inoculated at the same time as rabbit No. 8. This result may perhaps be explained by assuming that this guinea-pig was unusually resistant to the virus and its toxin. It is to be noted, too, that, in this instance, the injection was subcutaneous, not intravenous.

C. *Cultural Experiments and Inoculation of Cultures into Animals.*

In view of apparently positive results we had obtained in the transmission of influenza to animals by means of filtered materials,

blood, sputum, &c., from cases of influenza, it was decided to attempt to obtain cultures of the virus by means of the method used by Noguchi. We had always obtained uniformly negative results in attempting to culture different filtrates, using the ordinary media employed by us in investigating the bacteriology of sputum, post-mortem material, &c., from cases of influenza, i. e. serum broth, blood agar, heated blood agar, &c., but the Noguchi technique still remained to be tried.

In November 1917 Foster reported (*Journal of Infectious Diseases*, Vol. xxi, No. 5), that in studying the etiology of common colds he had been able to isolate an organism by means of Noguchi's methods, and that he had reproduced the disease in human beings by means of nasal instillation of his culture. His method and technique are described in great detail in his monograph, and we have followed them as closely as possible in a laboratory in the Field. Although the work is incomplete, we consider that the results are definite enough to warrant publication.

(a) *Preparation of Noguchi culture tubes.* At first we had great difficulty in obtaining a supply of ascites fluid, and our earlier experiments were made with human blood serum with negative results. This is not surprising as it is recognized that blood serum tends to inhibit the growth of filtrable organisms, that of poliomyelitis being an example.

Finally a case of general polyserositis occurred and a litre of clear amber-coloured ascites fluid was obtained and proved to be sterile.

Special tubes were obtained measuring 1 cm. × 20 cm. These were sterilized by hot air and filled with ascites fluid to within 6 cm. of the top. These tubes were then allowed to stand five days at 37° to eliminate the possibility of contamination.

A rabbit was now anaesthetized with ether and the neck vessels severed with a sharp bistoury and as much blood as possible allowed to drain away. It was then stretched out on a board and thoroughly drenched with strong iodine solution.

Scissors, forceps, and scalpels had been sterilized in three separate packets for use at different stages in removing the kidney of the rabbit.

The skin was incised in the mid line from the neck to the tail and carefully stripped back from the abdomen and thorax and fastened down to the board. The tissues were again drenched with iodine.

A semi-lunar line curving outward, about 2 cm. wide, was thoroughly seared from the costal margin to the groin.

Another packet of instruments was opened and the abdomen slit open along the seared line and the two edges of peritoneum held back with rat-tooth forceps by an assistant. Meanwhile another packet of instruments had been opened. The bowels were pulled through the opening with the hand covered with sterile gauze and the kidneys picked up with a pair of forceps and quickly removed with scissors and transferred to a sterile petri-dish.

The kidney was then cut up with sterile scissors into small cubes just large enough to enter the culture tube easily, the lid of the dish being held over the instruments by an assistant while this was being done.

The tubes were then opened one by one and a piece of kidney put into each with a hot platinum wire and allowed to fall to the bottom of the tube. Sterile liquid paraffin was then poured into each tube to within 2 cm. of the top, the tube was plugged, and a rubber cap put on.

These tubes were allowed to stand for one week before being used.

When cultures were made the material to be cultured was drawn up into a long teated capillary pipette and then expelled until it had formed a drop on the tip, when the pipette was quickly plunged through the paraffin and ascites fluid to the kidney and the material all expelled to within 3 or 4 cm. of the tip. The pipette was now withdrawn quickly and the tube plugged again.

(b) *Staining methods.* When it was desired to examine microscopically material from the bottom of the tube, this was obtained with a capillary pipette and the pipette broken off above any oil that might have clung to it. A few drops were then placed on a slide, spread slightly, and allowed to dry in the open air upside down.

They were fixed for one hour in pure methyl alcohol and stained for twenty-four hours in 5 per cent. Giemsa stain, always having the smear downward to prevent any stain from depositing.

Foster stated that one should see in uninoculated tubes in twenty-four to forty-eight hours only a clear zone of haemolysis above the kidney, and possibly a very faint opalescent zone, but nothing more, and we have confirmed this. Even the haemolytic zone may be very faint if the animal from which the kidney was removed had been thoroughly exsanguinated.

When we first commenced the cultural work in December 1918 we were temporarily somewhat short of animals. This fact led to our obtaining our first culture from the kidney of an infected animal instead of directly from the material from human cases. This came about in the following way :

Wishing to prepare some Noguchi tubes, and having no normal rabbits from which to obtain the tissues, we used the kidney from a rabbit that had been inoculated with a filtrate of influenza sputum and which had reproduced the characteristic signs in its lungs. The Noguchi tubes thus prepared were placed in the incubator to ensure their sterility.

In twenty-four hours a clear pale-red zone had developed above the tissue, and in forty-eight hours it was noticed that a faint cloud was appearing. In seventy-two hours this had increased, and a week later the cloud was about 3 cm. high and a fine deposit had begun to settle on the bottom of the tube.

The cloud showed no tendency to blend with the clear ascites fluid above and was rather more dense immediately above the kidney. The culture was examined by subculturing for the presence of any non-filtrable contamination, and this was found to be absent.

On the thirteenth day a smear was made and stained with 5 per cent. Giemsa stain for twenty-four hours after fixation in methyl alcohol for one hour. In this smear the following appearances were noted :

Numerous small coccoid bodies in size varying from about 0·1μ to 0·2μ and generally single, but often taking on a diplococcal arrangement and sometimes occurring in small agglomerations.

Some showed a rather delicate halo, the significance of which has not been determined.

With Giemsa, they usually stained a deep purple, but some which were apparently degenerate were paler in colour and of a pinkish tinge.

On this particular occasion the cocci was Gram-negative, but it has since been shown that in young cultures Gram-positive organisms may be found.

The cloudy material was transferred to other Noguchi tubes and the same phenomena occurred. Smears made from the resulting subcultures, stained with Giemsa, showed the same picture as described above.

(c) *Animal Experiments with Cultures.*

Experiment I. Original cultures were put up on December 15, 1918.

On December 28, when the cultures were thirteen days old, a mouse was inoculated subcutaneously with 0·25 c.c. of the original culture. It died six days later. Its lungs showed some haemorrhagic areas.

Post mortem. Its kidneys were removed with aseptic precautions and implanted in a Noguchi ascites fluid tube. A subculture was obtained, but not proceeded with farther. A rabbit was also inoculated intravenously with 1 c.c. of the culture. It was killed seven days later and, although the lungs of the animal did not show any typical signs of a marked infection, a culture was obtained from the rabbit's kidney.

In addition to these animal inoculations, subcultures of this virus were made on December 28 and a definite cloud was obtained. Fifteen days later, on January 12, 1919, these cultures were mixed together and the following animals were inoculated as shown in table:

Original Culture.

Rabbit (see text)
|
Mixed subculture
R. 2
15 days old.

Baboon 12.	Baboon 13.	Mouse.	Rabbit 11.	Guinea Pig 16.	Monkey 15.
+	−	+	+	+	+

Rabbit 12. (+) — Received 2 c.c. intravenously of filtered lung extract from Baboon 12. → Rabbit 14. (+) — 2 c.c. filtered lung extract from Rabbit 12.

Rabbit 13. (+) — 2 c.c. Intravenously unfiltered lung extract from Baboon 12.

Guinea Pig 16. (+) — Filtered / Unfiltered

Guinea Pig.	Guinea Pig.	Guinea Pig.
+	*	*

Died 15th day → Guinea Pig. *

\+ = A positive result.
* = Still under observation.

At the same time as the animals were inoculated the subculture Rabbit 2 was inoculated into another Noguchi tube. This culture produced the usual cloud and the smear showed the presence of the typical coccoid bodies.

Thus the third generation by direct subculture has been reached and at the time of writing it has not yet been taken farther.

Besides these experiments we have also succeeded in growing the organism: (1) From a filtered sputum from a case of influenza; this also has been carried through to the second generation. (2) From the filtered lung extract of Baboon 12. (3) From the kidney of another infected animal.

Controls. On the other hand, the kidneys from normal rabbits when cultured in Noguchi tubes have failed to produce these organisms on culture, as also the kidney from a monkey inoculated with sputum from a case of simple acute bronchitis.

V. Conclusions.

The number of experiments carried out by us is too small to justify the drawing of final conclusions. These experiments were brought to an end by the cessation of the epidemic, and the loss of laboratory attendants consequent on demobilization. We feel, however, that we are in a position to make the following deductions from our work:

I. The apparent immunity of some animals to filter-passing viruses and the occasional difficulty of the transmission of these viruses by means of blood is well known. When this is taken into account the number of positive results obtained by us would seem to be significant.

II. The pathological lesions in what may be called experimental influenza in animals closely resemble those seen in the lungs of men.

III. There is some evidence in favour of the view that the passage of the virus from one animal to another may raise its virulence.

IV. Inoculation of the filtered and unfiltered sputum taken from cases of influenza, especially at an early stage of the disease, has been found to produce lesions in the lungs in a high proportion of inoculated animals. The inoculation of blood may not always produce such striking results.

V. A minute coccoid micro-organism may be grown by Noguchi's cultural methods from:

(*a*) The kidney of infected animals;
(*b*) The filtrates of lung tissue; and
(*c*) The filtered sputum from cases of influenza.

The cultures have been carried by us to the third generation by direct culture. The cultures when inoculated into animals produce typical 'experimental influenzal' lesions, and cultures can be recovered again from the animals so inoculated, either by direct culture or by filtered extracts of the organs.

VI. In view of these findings we consider that there are very strong grounds for considering that:

(*a*) the organism isolated by us is capable of passing through a filter, and

(*b*) that it is in all probability the cause of influenza as seen to-day.

A similar organism, having the same properties, has already been described by Captain J. A. Wilson, R.A.M.C., working independently at No. 20 General Hospital.[3] We had completed our investigations before his research came to our knowledge. Since the publication of his work on this subject, we have shown him our preparations and he considers our organism to be the same as his own, in which opinion, after examination of his slides, we are in agreement.

We are indebted to the Medical Research Committee for providing the material for the work, to Colonel S. L. Cummins, C.M.G., A.M.S., Adviser in Pathology, British Armies in France, for valuable advice; to the O.C.s of No. 2 Stationary Hospital and No. 3 Australian General Hospital for allowing us the use of the laboratories attached to their hospitals, and to the O.C., No. 2 Stationary Hospital, for the use of his wards.

The help of Pte. Webster, A.I.F., and Pte. Whally, R.A.M.C., has been invaluable in the care of the animals, and our thanks are also due to Pte. Urquhart, R.A.M.C., our laboratory attendant, for much hard work in the preparation of media.

NOTE BY COLONEL S. L. CUMMINS,

Adviser in Pathology, British Armies in France.

While Major H. G. Gibson, R.A.M.C., was actually engaged in preparing the above Summary of the work of the Influenza Research Team, of which he was the Senior Officer, he was, himself, attacked by the disease in its severest form. He had been working for long hours in the laboratory at cultural and passage experiments with the organism believed to be the 'Filtrable Virus' of influenza, and the attack found him overdone and weary from his self-forgetting efforts to solve a problem of pressing military and general importance.

His death, a grievous loss to Medical Science and to the Royal Army Medical Corps, was still such an end as a soldier would have chosen. Laborious tasks cheerfully undertaken and dangers resolutely faced are no less glorious in the laboratory than in the trenches.

Major Gibson had no opportunity of revising or even finishing his Summary, which has had to be completed from his notes and records. Any obscurities or redundancies are therefore to be attributed, not to him, but to the sad circumstance that others had to arrange his work for publication.

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2. THE BACTERIOLOGICAL FLORA OF THE RESPIRATORY TRACT IN CASES OF INFLUENZA

BY

Major H. G. Gibson, R.A.M.C.

AND

Major F. B. Bowman, C.A.M.C.

Although careful studies have been made of the sputum and material from the naso-pharynx in cases of influenza in widely separated areas, these have in many instances been contradictory. It was decided to investigate this problem in the series of cases being studied clinically for this report. The findings will be found briefly under the descriptions of the individual cases. However, it may be of interest to make a few general remarks regarding the work done.

In many cases of influenza it was impossible to obtain a specimen of sputum in the first few days of the disease and from these only a swab from the throat was made.

I. Methods.

A. *Culture Media.*

For all primary cultures human blood agar plates were used. To be of any practical value as regards the presence or absence of haemolysis they must be made very carefully. Ordinary plain agar with a reaction of about plus six is prepared and kept in stock. When plates are to be made the flask of agar is melted and the temperature reduced to 50°, when defibrinated human blood is added in the proportion of 5 per cent. and well mixed, and plates are then poured. Defibrinated blood is preferable to citrated blood, and the serum should not be removed as this aids in a clear-cut haemolytic reaction. Care should be taken that the agar is not too hot when the blood is added, as some alteration in the cells occurs which interferes with haemolysis. Any carbohydrate in the medium may mask the result by the production of acid with a resulting brown coloration.

For subcultivation of *B. influenzae* the following medium was used. This was first recommended by Fitzgerald (*Centralbl. f. Bakteriol*, 56, 464). Flasks of agar are prepared as above and defibrinated human blood added in the same proportion and the whole brought to a temperature of 80°, when the medium assumes a homogeneous non-granular chocolate colour. On this medium the organism grows profusely.

Levinthal's medium is also of value, and has the advantage of being clear ; but often a batch, although made very carefully, will fail to grow the organism, and for this reason we gave up its use entirely.

Fildes states that this difficulty may be obviated by the use of glass wool in filtering (*Lancet*, November 23, 1918).

For stock cultures of *B. influenzae* (Pfeiffer) a little defibrinated blood is added to a meat broth tube and heated to 80° C. Transplants are not necessary more often than once a month using this method. (This was recommended by Captain Perry, R.A.M.C., and Captain Fleming.)

In an attempt to obtain a clear medium for the growth of *B. influenzae* and streptococci the following method was used, and although it failed in so far as a profuse growth of *B. influenzae* was concerned, a rather interesting phenomenon occurred.

A 1 per cent. solution of saponin was made in distilled water and defibrinated blood was added to this until no more dissolved, when a very concentrated solution of haemoglobin resulted which was almost black in colour. This was added in different amounts to agar, but the results were not encouraging so far as haemolytic reaction of streptococci were concerned, although *B. influenzae* grew quite well. In attempting to find a clear fluid medium which would differentiate between so-called green or brown haemolysis and clear haemolysis, a little of this ' saponized ' blood was added to plain broth tubes until they assumed a pale cherry red colour. Cultures of streptococci were seeded into this medium and it was found that those belonging to the *viridans* group could be separated from the haemolytic group quite easily. *Streptococcus viridans* produced a definite dirty green colour in marked contrast to the haemolytic group, where little change in colour was noted but a profuse growth occurred.

We also found that this concentrated haemoglobin solution when added to agar and heated to 80° C. produced a useful medium for the growth of *B. influenzae*.

For the preparation of carbohydrate media for the classification of streptococci see below under blood cultures.

II. Material Examined.

A. *Sputum*.

This was obtained from the patient in a clean vessel. If possible it was collected for twenty-four hours.

We have found that samples from true cases of influenza have a characteristic appearance and from this alone we almost could foretell the presence or absence of *B. influenzae*. It usually appeared rather homogeneous and creamy and not in large greenish white lumps and flakes. In colour it was pale greyish yellow with sometimes a faint rusty tinge. It was extremely tenacious and elastic, and when picked up with a wire stretched out into long threads.

A small globule of sputum about the size of a pea was transferred to a petri dish and washed thoroughly in several changes of distilled water, any superfluous water being absorbed by strips of blotting paper. The sputum was then torn into small fragments with forceps, and one of these was transferred to a blood agar plate and carefully streaked with a platinum wire. Another small portion was smeared on slides for staining by Gram's method, and for capsules.

B. *Naso-Pharyngeal Swabs.*

In making cultures from throats sterile West swabs were used. These were smeared carefully on one side of a blood agar plate and streaks were made from side to side with a platinum wire.

C. *Post-Mortem Material.*

Bronchial fluid. The trachea and lungs having been removed entire, the trachea was carefully opened and the lungs lifted up and any material flowing past the bifurcation caught in a test-tube and taken to the laboratory. Here smears and cultures on blood agar were made as was done with the sputum.

Tracheal scrapings. The trachea was wiped dry with gauze and the inflamed mucous membrane scraped with a knife and the material transferred to a test-tube. It was then examined fresh and cultures made.

Lung cultures. A portion of lung was taken to the laboratory and seared and cultures made with a loop on to blood agar.

Pericardial fluid and heart's blood. Were removed with sterile pipettes after searing and taken to the laboratory for culture into serum broth and on to blood agar plates.

Any other material was treated in the same way.

III. Results of Bacteriological Examination.

It is interesting to note that early in this epidemic we had some difficulty in the preparation of our carbohydrate media, and until this was adjusted organisms were described as pneumococci or streptococci simply on morphological grounds and the reaction on blood agar. The result was that some cases were reported as having pneumococci in the sputum when, as shown by our later work, the organism was probably a streptococcus. The cultures and smears taken from post-mortem material were noted in the same way.

The marked pleomorphism shown by certain organisms even in fresh smears made observations very misleading. In certain specimens of sputum examined, typical pneumococci (capsulated), diplostreptococci (with capsules), and smaller streptococci were found. On culture only one type of colony appeared. This was a rather dry crater-like flat colony sometimes with irregular edges, showing no haemolysis and with a certain amount of greenish-brown coloration around it. Smears in twenty-four hours showed it to be a lanceolate diplostreptococcus, and the same colonies examined in four days showed extremely marked pleomorphism, cocci large and small, clubbed forms, flattened and round streptococci were observed and in fact almost every form imaginable. Thus it can be easily seen that from morphology alone it would be utterly impossible to classify organisms as pneumococci or streptococci as seen in stained specimens of sputum. The same statement would apply to material removed from other sources where the same organisms were found.

Material obtained from twenty-four post-mortem examinations was examined bacteriologically by methods cited elsewhere.

Bronchial Fluid : 25 Cultures.			
B. influenzae (Pfeiffer)	17	times or	68%
Streptococcus viridans	22	,,	88%
Haemolytic streptococcus (not associated with *B. influenzae*)	4	,,	16%
Lung : 19 Cultures.			
B. influenzae (Pfeiffer)	6	,,	32%
S. viridans	16	,,	84%
Haemolytic streptococcus	3	,,	16%
Pericardial Fluid : 20 Cultures			
B. influenzae (Pfeiffer)	0	,,	
S. viridans	1	,,	5%
Haemolytic streptococcus	3	,,	15%
Heart's Blood : 6 Cultures.			
S. viridans	1	,,	17%
Tracheal Scrapings : 13 Cultures.			
B. influenzae (Pfeiffer)	11	,,	85%
S. viridans	12	,,	90%
Haemolytic streptococcus	1	,,	8%
Tracheal Glands : 10 Cultures.			
All negative with the exception of 1 where a haemolytic streptococcus was isolated, but this was found in all other material examined from this case			10%
Pleuritic Fluid : 5 Cultures.			
B. influenzae (Pfeiffer)	1	,,	20%
Haemolytic streptococcus	1	,,	20%
S. viridans	2	,,	40%

From a post-nasal abscess in one case a profuse growth of *B. influenzae* resulted, accompanied by *S. viridans*.

Six subclasses of streptococci were found in our investigation of thirty-six stains (see Table II for fermentative reaction of these).

For classification of thirty-six stains of streptococci showing sources from which they were obtained see Table III.

26 or 72 per cent. belonged to the *viridans* group and of these 58 per cent. gave the reactions of *Streptococcus mitis*.

The absence of pneumococci from our cultures is remarkable in view of the work of others. By some workers the pneumococcus has been described as the predominant organism found in the material examined. We were led astray as stated before, early in our work, by identifying organisms on morphology alone, but later when ' typical ' pneumococci were found they were tested for bile solubility and put up against standard type agglutinating sera and in only two instances were the original classifications confirmed.

From twelve specimens of sputum examined, *B. influenzae* (Pfeiffer) was isolated eight times (see Table V) and always associated with a streptococcus of the *viridans* group in numbers that would suggest that they might possibly have played some part in conjunction with *B. influenzae* in the causation of secondary infections.

The question of symbiosis of *B. influenzae* with other organisms has been suggested by different workers. We found that it grew profusely with a streptococcus of the *viridans* group and was still alive after two weeks at room temperature. There seems little doubt that symbiosis exists and may account for the number of times the organism has been grown easily in association with the peculiar streptococcus mentioned above.

The following experiment rather graphically illustrated this fact. Streaks from an emulsion of Pfeiffer's bacillus were made on a blood agar plate about a centimetre apart, and at right angles to these streaks were made with a culture of *S. viridans*. Where the lines crossed it was found that Pfeiffer's bacillus had grown much more profusely than along the streaks.

IV. Results of Blood Culture in Twenty Cases of Influenza.

Blood cultures were made from the twenty cases of influenza studied clinically for this report.

The technique employed was the following:

Media Employed.

(a) *Human blood agar.* (For preparation see under methods previously described.)

(b) *Glucose serum broth* was prepared with horse serum. This was obtained by bleeding horses from the jugular vein into sterile Winchester quart bottles and allowing the blood thus obtained to clot. The serum was then drawn off and an equal quantity of normal saline was added, after which the mixture was passed through a Doulton filter.

The glucose broth was made up according to the formula quoted below after that given by Holman for his carbo hydrate serum broth.

Double strength trypsin broth prepared according to Douglas' method . . . . . (+12 acid)	200 c.c.
Distilled water	100 c.c.
Glucose	4 gram.

Flasks of this medium were prepared, each containing 25 c.c., and to each of these was added 16·5 c.c. of the diluted and filtered serum.

The patients were bled from one of the veins at the bend of the elbow and 2 c.c. of blood were sown into the flasks of serum broth, also a drop of blood was smeared on the surface of blood agar.

In some cases the glucose serum broth was incubated both aerobically and anaerobically, but in no instance was a growth obtained under anaerobic conditions when a negative result was obtained in aerobic culture.

Table I shows the day of disease in each case in which blood cultures were made and the number of positive results obtained.

The cases in which only one or two observations were made were cases which were of a milder nature and rapidly recovered.

Three cases out of twenty gave positive results with blood culture. Two of these positive results were obtained within a short time of death, that is in Cases Nos. 1 and 3, the first dying on the seventh day and the second on the fifteenth day. In both these cases the preliminary cultures proved sterile, and Case No. 18 was the only one in which a positive blood culture was obtained any length of time before the fatal termination of the illness.

Table I.

Days of Disease.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	*Final disposal of Case.*
Case No. 1				—			+ ‡														Died
,, 2				—																	Recovered
,, 3				—		—			—				+		‡						Died
,, 4					—																Recovered
,, 5					—							—								—	,,
,, 6				—																	,,
,, 7	—																				,,
,, 8			—																		,,
,, 9				—																	,,
,, 10						—					—					‡					Died
,, 11				—																	Recovered
,, 12		—																			,,
,, 13						—					—				—				‡		Died
,, 14				—																	Recovered
,, 15			—						—		‡										Died
,, 16				—	—																Recovered
,, 17								—													,,
,, 18		+			—																,,
,, 19		—																			,,
,, 20		—		—																	,,

‡ Patient died on this day.

The organism recovered was in each case a Gram-positive diplostreptococcus which grew on blood agar as small flat crater-like colonies producing no haemolysis, but imparting a brownish green tinge to the media.

They showed extreme pleomorphism in cultures which were a few days old.

In glucose broth they produced a uniform turbidity with a granular deposit. None were bile soluble.

Following Holman's formula the streptococci isolated all belonged to the *viridans* group, that from Case No. 1 giving the reactions of *S. faecalis* and those from Cases Nos. 3 and 18 of *S. mitis*.

In no case was *B. influenzae* recovered from the blood stream.

From these thirty-two attempts at blood culture it would appear that streptococci rarely enter the blood stream in the early stage of the disease, and usually their presence there may be taken as a terminal phenomenon.

In Case No. 18 a streptococcus was recovered on what was apparently the second day of the illness, but the patient may have had the disease for a few days previous to this. The organism was not recovered at a second attempt.

V. Agglutination Reactions.

In view of the fact that *B. influenzae* (Pfeiffer) has been found constantly in the sputum of cases of influenza and in material removed *post mortem*, the question of specific agglutination arises, and this was studied in twelve cases.

These were selected at different stages in the course of the illness from two to twenty-five days.

The organism was grown on heated blood agar, washed off with 0·85 per cent. saline, and any extraneous matter allowed to settle. The supernatant suspension was then decanted, well shaken, and diluted to pale opalescence with saline.

Several methods of performing reaction were tested.

1. Using fresh suspensions of *B. influenzae*.
2. Using carbolized suspensions.
3. Using formalinized suspensions.

The suspension with serum in different dilutions were placed in the water bath at 37° C. and 55° C.

The only well-defined results were obtained with formalinized cultures in the water bath at a temperature of 55° C.

By consulting Table VI the result may be seen briefly.

The most marked reactions were obtained between the second and sixth day inclusive, and the strongest reaction of all was obtained in a second-day case.

It might be noted also that in some instances the higher dilutions gave the most marked agglutination reactions, although these were not continued to the limit of agglutinability.

VI. Summary.

The bacteriological investigations of cases of influenza were begun before we had seen the work of Nicolle and Lebailly, and although

there seems to be little doubt that none of the primary symptoms in this disease are caused by any of the organisms described in this section, their importance as secondary invaders cannot be denied. For this reason the work was continued after we had obtained positive results in our animal experiments with filtered material from cases of influenza.

The following conclusions may be cited briefly :

(1) *B. influenzae* (Pfeiffer) is usually present in the respiratory tract of cases of influenza. It was found in 67 per cent. of specimens of sputum and somewhere in the respiratory tract of 80 per cent. of cases examined *post mortem*. Most commonly it was found present in material scraped from the inflamed trachea and in fluid welling up into the larger bronchi. In haemorrhagic areas in the lungs it was seldom found. It was isolated once from pleural exudate, and in almost pure culture from a post-nasal abscess in one instance. Blood cultures were uniformly negative in regard to this organism as well as cultures from the heart's blood and pericardial fluid.

(2) Following Holman's formula for the classification of streptococci, we have found commonly present a diplostreptococcus, showing marked pleomorphism and belonging to the *viridans* group. In 88 per cent. of examinations of post-mortem material it was found, and equally common in the bronchial fluid, tracheal scrapings, haemorrhagic areas in the lungs, &c. Morphologically, at times it might have been called a pneumococcus, but agglutination with type sera and tests for bile solubility disproved this.

(3) Haemolytic streptococci were very uncommon in our series. Only five strains were isolated from twenty-five post-mortems.

(4) A symbiotic relationship between *B. influenzae* and *Streptococcus viridans* seems probable.

(5) Agglutination reactions as regards *B. influenzae* show that the most marked reaction with patients' serum occurs between the second and sixth day of the disease. Agglutination may be more marked in the higher dilution than in the lower.

(6) Agglutinative reactions with patients' serum and an emulsion of *Streptococcus viridans* were uniformly negative.

(7) The absence of pneumococci in our series seems remarkable in view of the work of others and the possibility of the preponderance of certain organisms in certain areas is worthy of consideration.

Table II.

Streptococci Isolated by us from Cases of Influenza Classified according to Holman.

\+ = fermentation or haemolysis.

Organism.	*Lactose.*	*Mannite.*	*Salicin.*	*Haemolysis.*	
S. mitis	+	—	+	—	*Viridans* group.
S. faecalis	+	+	+	—	*Viridans* group.
S. salivarius	+	—	—	—	*Viridans* group.
S. anginosus	+	—	—	+	Haemolytic group.
S. haemolyticus ii	—	+	+	+	Haemolytic group.
S. pyogenes	+	—	+	+	Haemolytic group.

Table III.

Thirty-six Strains of Streptococcus Typed according to Holman.

Source.	*S. mitis.*	*S. faecalis.*	*S. salivarius,*	*S. anginosus.*	*S. haemolyticus* ii.	*S. pyogenes.*
aso-pharynx . .	2	1	1	2	1	..
putum	1	1	2	1	..	2
racheal scraping . .	1	1	..	..	..	1
ronchial fluid . .	4	2	2	..	..	2
ung	3	..	..	..	..	..
leural exudate . .	..	..	..	..	1	..
eart's blood. . .	..	..	..	..	..	..
ericardial fluid . .	1	..	..	..	1	..
racheal gland . .	..	..	..	..	1	..
lood culture . .	2	1	..	..	..	..

Table IV.

Bacteriology of Twenty-five Post-mortem Examinations.

P. = *B. influenzae* ; V. = *S. viridans* ; H. = *S. haemolyticus* ; O. = No observation made.

P.M.	*Bronchial fluid.*	*Lung.*	*Tracheal scraping.*	*Tracheal gland.*	*Heart's blood.*	*Pericardial fluid.*	*Pleural exudate.*
1	P.V.	P.V.	O.	O.	O.	O.	O.
2	H.	H.	O.	O.	O.	O.	O.
3	P.V.	V.	O.	O.	O.	O.	O.
4	P.V.	V.	O.	O.	O.	O.	O.
5	V.	V.	O.	O.	O.	O.	O.
6	P.	O.	O.	O.	O.	O.	O.
7	P.V.	O.	O.	O.	O.	Neg.	O.
8	V.	O.	O.	O.	O.	Neg.	O.
9	P.V.H.	P.V.H.	O.	O.	O.	H.	O.
10	H.	O.	P.	H.	O.	H.	O.
11	P.V.	Abscess P.V.	O.	Neg.	O.	V.	O.
12	V.	O.	V.	Neg.	O.	V.	O.
13	V.	V.	P.V.	Neg.	Neg.	Neg.	O.
14	P.V.	V.	P.V.	Neg.	O.	Neg.	O.
15	P.V.	V.	P.V.	Neg.	O.	Neg.	O.
16	P.V.	V.	P.V.	Neg.	O.	Neg.	O.
17	V.	V.	P.V.	Neg.	O.	Neg.	O.
18	P.V.	Neg.	P.V.	Neg.	Neg.	Neg.	Neg.
19	P.V.	Staph.	V.	Neg.	O.	Neg.	Neg.
20	P.V.	V.	P.V.	Neg.	O.	Neg.	Neg.
21	P.V.	P.V.	P.V.	O.	Neg.	Neg.	P.V.H.
22	P.V.	V.	*M. catarrhalis* (pure)	O.	Neg.	Neg.	O.
23	V.H.	P.V.H.	P.V.H.	O.	Neg.	Neg.	O.
24	P.V.	P.V.	P.V.	O.	Neg.	Neg.	O.
25	P.V.	O.	O.	O.	O.	Neg.	V.

Table V.

Bacteriology of Sputum from Twelve Cases of Influenza taken between the First and Fourth Days of the Disease.

Case.	1	2	3	4	5	6	7	8	9	10	11	12
influenzae . .	+	+	—	+	+	—	+	—	+	—	+	+
viridans . .	—	+	1	+	+	+	+	+	+	+	+	+
haemolyticus .	—	+	—	—	—	—	—	—	+	—	+	—
Gram-negative Diplococci * . .	+	+	—	—	+	+	+	—	+	+	—	—

* Unfortunately we were without type agglutinating sera for meningococci for some time, and the organisms found are classified as Gram-negative diplococci.

Table VI.

Agglutination Reactions.

Case.	*Day of Disease.*	*Serum Dilution.* 1/20	1/40	1/80	1/160	1/320	1/640
We.	2nd	+	++	+	+	+	±
Pi.	,,	±	±	+	+	±	±
Pr.	,,	++	+++	+++	++	++	++
D.	6th	+	+	+	++	++	++
B.	,,	++	++	0	++	++	+
J.	,,	+++	++	+	+	±	+
Ch.	7th	+	+	—	+	—	—
Wa.	12th	—	—	—	—	—	—
Gr.	16th	—	—	—	—	—	—
Fa.	20th	—	—	—	—	—	—
Ca.	16th day after relapse	±	—	±	+	+	++
Tu.	25th	—	—	—	—	—	—

3 (*a*). A STUDY OF TWENTY CASES OF INFLUENZA, CLINICALLY

By Major C. E. SUNDELL, R.A.M.C.

I. Source of Material.

The cases upon which this report is based were drawn from those admitted to this hospital during October and November 1918.

Two series are included :

(1) Special—twenty cases in which full pathological records are available for correlation with the clinical findings.

(2) General—1,000 cases in which, though investigations were incomplete, certain points of interest were considered in detail.

The patients in both series include British officers and other ranks and German prisoners of war : in the general series the large majority was made up of German prisoners of war.

II. Types of Case.

These were trivial, mild, or severe. It was noteworthy that the proportion of severe cases was at its height during the middle third of the epidemic, and that the severity of the disease diminished considerably before the actual incidence of the disease itself diminished.

III. Age of the Patient.

The population from which our patients were drawn was composed of men between 19 and 50—most of them between 20 and 35. It appeared that (1) the disease was much more prevalent among the young and (2) the severe type of the disease was much more prevalent among the younger than among the older men.

IV. Clinical Outline of the Disease.

As observed here, the disease presented certain characteristic features, viz. :

(1) Suddenness of onset.
(2) Severity of prostration.
(3) Constant involvement of the respiratory tract.
(4) High fever.
(5) Relatively slow pulse.
(6) Danger of rapid development of fatal complications.
(7) Frequency of psychical disturbance.
(8) Tendency to relapse.

V. Clinical Features of the Disease.

A. *Mode of Onset.*

This was always rapid, often sudden. Some men complained of malaise for several days before the severity of their symptoms com-

pelled them to come to hospital ; such cases on investigation were found to have been either originally mild cases which had developed complications or to be relapses. When treatment was sought early the onset was always described as sudden, with severe frontal headache, sometimes vomiting, fainting, usually with severe prostration, and often with shivering. We were impressed by the frequency with which patients coming under treatment late developed the disease in a severe form.

B. *Temperature.*

In uncomplicated cases the temperature reached its highest level during the first forty-eight hours. It remained at this level with remissions of 1° to 2° for twenty-four to forty-eight hours and fell by lysis with steep peaks reaching normal about the fifth day. Relapses two or three days later of slighter severity and shorter duration were not uncommon ; they recalled the intermittent pyrexia of trench fever. The original fall of temperature in a minority of the cases was by crisis.

C. *The Pulse.*

Relative slowness of the pulse was a striking and common feature. This lack of correspondence between the pulse and temperature curves was seen while the fever was at its height but before serious complications became manifest. As a signal of their imminence or onset a sudden rise in the pulse-rate was of great significance.

D. *Alimentary System.*

Tongue. In the majority of cases the tongue presented the appearance usually associated with a febrile disease, with the usual thick dry fur while the fever was maintained, and cleaning in the usual way when defervescence was established. Frequently, however, a peculiar mauve tint was seen on the dorsum and under-surface associated with a similar coloration of the fauces and lips. This appearance suggested a severe type of case : it was seen typically in ' toxaemic ' cases, and its appearance during the course of the disease was of ill omen. A return to normal colour of the tongue was an early sign of convalescence.

Stomach. Gastric disturbance was very common. Vomiting in the early stages has been seen frequently ; it usually ceased during the first forty-eight hours in the absence of complications or the excessive use of stimulating expectorants. Intractable vomiting persisting for several days was not uncommon in severe cases and its persistence after the first two days was regarded as a danger-signal. Haematemesis was not seen.

Bowels. Diarrhoea, often associated with vomiting, was common in the first forty-eight hours. During convalescence lienteric diarrhoea occasionally developed. Melaena occurred in three patients ; in one of these the quantity exceeded a pint ; in all it arose between the seventh and tenth days in cases of great severity but which ultimately recovered completely. Tympanites was not uncommon.

Liver. A mild degree of jaundice was seen in a few cases ; it

never lasted more than seven days. The cases were slight and recovered quickly. Tenderness over the liver was not complained of.

Spleen. No enlargement was detected. Pain in the splenic region was occasionally complained of, but no local physical signs could be found to explain its origin. Post-mortem examination occasionally revealed the presence of slight recent splenic enlargement.

E. *Respiratory System.*

Some degree of involvement of the respiratory tract was constant.

Sore throat at the onset was a very common complaint; no abnormality beyond redness of the pharynx with occasional oedema of the fauces was apparent at this stage.

Coryza and '*cold in the head*' very rarely occurred.

Epistaxis has been very common: usually only one nostril was involved: the flow was sometimes considerable and recurred on slight irritation: it was most common about the fifth day of illness; if occurring later in a case apparently progressing well it seemed to be an indication of commencing relapse or the development of a more serious phase.

Substernal pain varying from a mild soreness to acute pain was almost invariable; in association with this discomfort tender spots were commonly found in the skin over the first and second intercostal spaces.

Chest pain on breathing was not a feature of the uncomplicated cases, but was sometimes one of the earliest signs of pulmonary complication.

Cough. All these patients had a cough. In the early stages it was dry, 'tickling', and rapidly becoming painful. As the disease progressed there was a tendency for the cough to become paroxysmal. In the final stages of a 'pneumonia' case it sometimes ceased altogether.

Sputum. Much could be learnt of the progress of the case from an examination of the sputum. In the first stages it was scanty, clear, white, viscid. Later it became abundant, frothy, liquid, and often blood-stained. When pulmonary complications supervened its characters altered accordingly.

Haemoptysis was common in cases of moderate severity. It was bright, streaky, usually scanty, but in a few cases profuse. It was most common between the fourth and seventh days of illness. Its occurrence later with commencing pulmonary involvement was of serious significance.

Respiration-rate in uncomplicated cases was very little altered.

Aphonia was common, sometimes persisting throughout the course of the disease. It occasionally developed late or during convalescence.

Physical signs in uncomplicated cases were very slight. Movement was unimpaired, vocal fremitus unaltered. Breath sounds were weak in certain areas, usually over one lower lobe, and moist inspiratory crepitations were always to be found: low-pitched rhonchi were common, especially in the axillary region.

(The signs of pulmonary complication are discussed under that heading.)

F. *Circulatory System.*

Blood-picture. Slight leucopaenia with a striking decrease of eosinophile cells was the rule.

Blood-pressure. Little change was detected: a tendency to reduction of 'pulse-pressure' was noted in a few cases. No prognostic significance could be attached to blood-pressure readings. Dicrotism was not noticeable.

Pulse frequency. A striking feature has been relative slowness of the pulse during the course of the disease: a pulse-rate of 80–90 with a temperature of 102°–104° has not been uncommon. With the onset of serious complications a rapid rise in the pulse-rate has been an important and common feature.

Pulse rhythm. Arrhythmia during the height of the disease has not been noted. In the late stages of fatal cases intermittency has been common. During convalescence rhythmic irregularity associated with respiratory movements has often been found.

Heart. Evidence of myocardial distress, as shown by praecordial pain, dilatation, and alteration of the heart-sounds, was found only in cases presenting toxaemia or pulmonary complications.

Pericardial effusion was commonly found at post-mortem: it was never sufficiently large to give rise to definite signs during life.

No case of *Endocarditis* occurred in our series.

Cyanosis. A peculiar violaceous tint of lips and tongue has already been mentioned; spectroscopic examination of the blood from one of these cases revealed no abnormal absorption-bands. This tinting was most common in toxaemic cases, but was not entirely confined to those of great severity.

Thrombosis and *Embolism* have not been seen.

Haemorrhage. The disease was associated with a haemorrhagic tendency. No part of the body was exempt. Epistaxis and haemoptysis were very common; melaena was not rare, intramuscular haemorrhages were occasionally seen; petechial rashes occurred; petechiae on lungs and pericardium were found in most of the fatal cases; large haemorrhagic areas in the lung were common; blood cells were frequently present in the urine.

G. *Renal System.*

One case presented all the classical signs and symptoms of acute nephritis at the onset. The renal condition cleared up in ten days and, although the case ran a prolonged course, did not recur.

Albuminuria and the presence of a few renal cells and blood corpuscles have been common in the febrile stage of the disease, but renal symptoms have been absent.

H. *Nervous System.*

The chief nervous manifestations have been psychical. Profound mental inertia with intense physical prostration has been an early feature in many cases. Delirium has been very common: it has usually been worse at night. In its most common form it has been sudden in its onset, brief in its duration, sudden in its relief, and

frequent in its recurrence. It has varied from mere confusion of ideas through all grades of intensity up to maniacal excitement. A common feature of the illusions has been their pleasant nature: many of the severest cases have enjoyed a state of complacency or sense of well-being which was entirely out of keeping with the seriousness of their condition.

Depression has been substituted for this mental exaltation in a few cases throughout the course of the disease and it has been common during convalescence; in a few instances this depression has merged into melancholia.

Sleeplessness has been a very common and very obstinate symptom.

Sensory and Motor changes have not been observed, with the exception of one patient in whom a transient brachial monoplegia with aphasia developed: no explanatory signs were found in the brain *post mortem*.

I. *The Skin.*

Colour. Bright flushing of the cheeks was common at the commencement of the disease. The violaceous or heliotrope tint of the lips, which has already been referred to, sometimes was seen also upon the skin of the cheeks and neck.

Sweating has been a very striking feature; it has occurred copiously in cases from whom all drugs had been withheld. Profuse spontaneous sweating seemed to be an indication that the disease was about to be successfully overcome.

Rashes. Herpes of the lips has not been common: it was seen chiefly in the presence of severe pulmonary complications.

Urticaria was seen occasionally; it was widespread and the wheals were of large size.

Sudamina were quite common.

Petechial rashes occurred in three cases; two of these were fatal, the third made a slow but complete recovery. In one case the rash was very profuse and covered the whole of the dorsal aspect of the trunk and limbs; it spread on to the flanks but did not involve the face or anterior surface of the trunk. This patient died on the fifth day of illness with severe pulmonary lesions.

J. *Muscles.*

Pain in the large muscles has been a constant symptom in the early stages: warmth has given relief: movement has increased it. Pain and rigidity of the abdominal muscles has occasionally been so great as to suggest ' an acute abdomen '. Haemorrhage into the rectus abdominis was obvious in two out of our sixty-two post-mortem cases.

K. *Subcutaneous Emphysema.*

One case has occurred in which spontaneous surgical emphysema spread from the neck to the groin. The case was fatal, but no perforation in the air passages was discovered. In three other cases mediastinal emphysema was discovered at autopsy.

VI. Complications or Sequelae.

Two forms call for notice :

(A) Toxaemic.

(B) Pulmonary.

Both have been very serious and between them they have accounted for all our fatal cases.

A. *Toxaemic Complications.*

As a rarity the case has sometimes shown profound toxaemia from the commencement. Our most rapidly fatal case died on the third day of his illness. More frequently the evidence of severe intoxication has not appeared till the third or fourth day. Certain signs and symptoms seemed to be of grave significance, viz. :

(1) Early cyanosis.
(2) Early delirium.
(3) Sudden rise in pulse-rate.
(4) Late onset or persistence of vomiting.
(5) Epistaxis commencing after the fifth day.
(6) Obstinate constipation.

The mortality among these patients has been high, death usually occurring between the third and ninth days. Reaction to treatment has been disappointing. Early venesection (30 ounces) seemed to do good in a few cases ; if delayed beyond forty-eight hours after the appearance of grave symptoms it was useless.

Signs of commencing recovery were :

(1) A fall in the pulse-rate.
(2) Decrease of delirium.
(3) Cleaning of the tongue.
(4) Copious sweating with a fall of pulse-rate.

Blood-pressure readings gave no clue to prognosis in these cases.

B. *Pulmonary Complications.*

Some degree of trachitis was found in every case. The pulmonary complications which arose were :

(1) ' Oedema '.
(2) Broncho-pneumonia.
(3) Pulmonary haemorrhage.
(4) Pleural effusion.
(5) Bronchiectasis or abscess of lung.

The onset of lung-changes were indicated by :

(1) Alteration in the character of the sputum.
(2) Increase in the respiration-rate.
(3) Pain in breathing.
(4) Diminished respiratory movement.
(5) Diminution of air-entry.

When the complicating condition was well established the diagnosis seldom gave rise to difficulty except in the case of massive ' oedema ' or ' gelatinization ' of the lung, which was sometimes confused with pleural effusion.

Here the early or warning signs only are discussed.

The Sputum. Serious significance was attached to the following changes in the sputum :

(1) Great increase in quantity with the appearance of abundant liquid froth.

(2) Pink staining of the frothy sputum.

(3) Appearance of abundant pellets of thick, curdy muco-pus.

Rapid increase in the respiration-rate was sometimes the first indication of serious pulmonary involvement.

Pain on breathing—probably pleuritic in origin—occasionally gave the first sign.

Diminution of respiratory movement was found to be an important early sign in many cases.

Diminution of air-entry was found to be second in importance only to changes in the sputum as an indication of commencing pulmonary change. Temporary weakening of the respiratory murmur over certain areas of the lung, especially at the base, was very common at an early stage of even mild cases of influenza, but any extension of these quiet areas was found to be an important sign of lung-involvement.

' *Oedema of the lung.*' The macroscopic and microscopic characters of this condition are described elsewhere. Clinically it was found to be local or general : sometimes the upper lobes only were involved ; more often the condition was present in other parts of the lung as well. The physical signs associated with it were diminished movement, impaired percussion-note, decreased fremitus, weak breath-sounds, and abundant fine and medium inspiratory and expiratory râles. A few instances occurred in which the dullness was so great and the respiratory sounds so muffled that the presence of a considerable effusion was erroneously suspected.

The onset of this condition was sometimes within the first twenty-four hours ; more often it developed suddenly about the third or fourth day. It was not associated with oedema elsewhere, and there was nothing to suggest that it was cardiac or renal in origin. It seemed probable that the condition was a direct pulmonary manifestation of the action of the ' influenzal ' agent ; it did not appear comparable to the oedema of the lung associated with renal or cardiac disease.

Consolidation of the lung was common ; it was always lobular in origin, though often massive in its distribution. No case of true croupous lobar consolidation occurred in our series.

Pleural effusion was very common. Usually moderate in amount, it showed a great tendency to recur after aspiration. Empyema was common, though in many instances the purulent nature of the effusion was not suggested by the character of the temperature chart. It was found necessary to explore every case of pleural effusion, and a surprising number of unsuspected empyemata were thus revealed.

Nervous sequelae were occasionally observed. Depression was common.

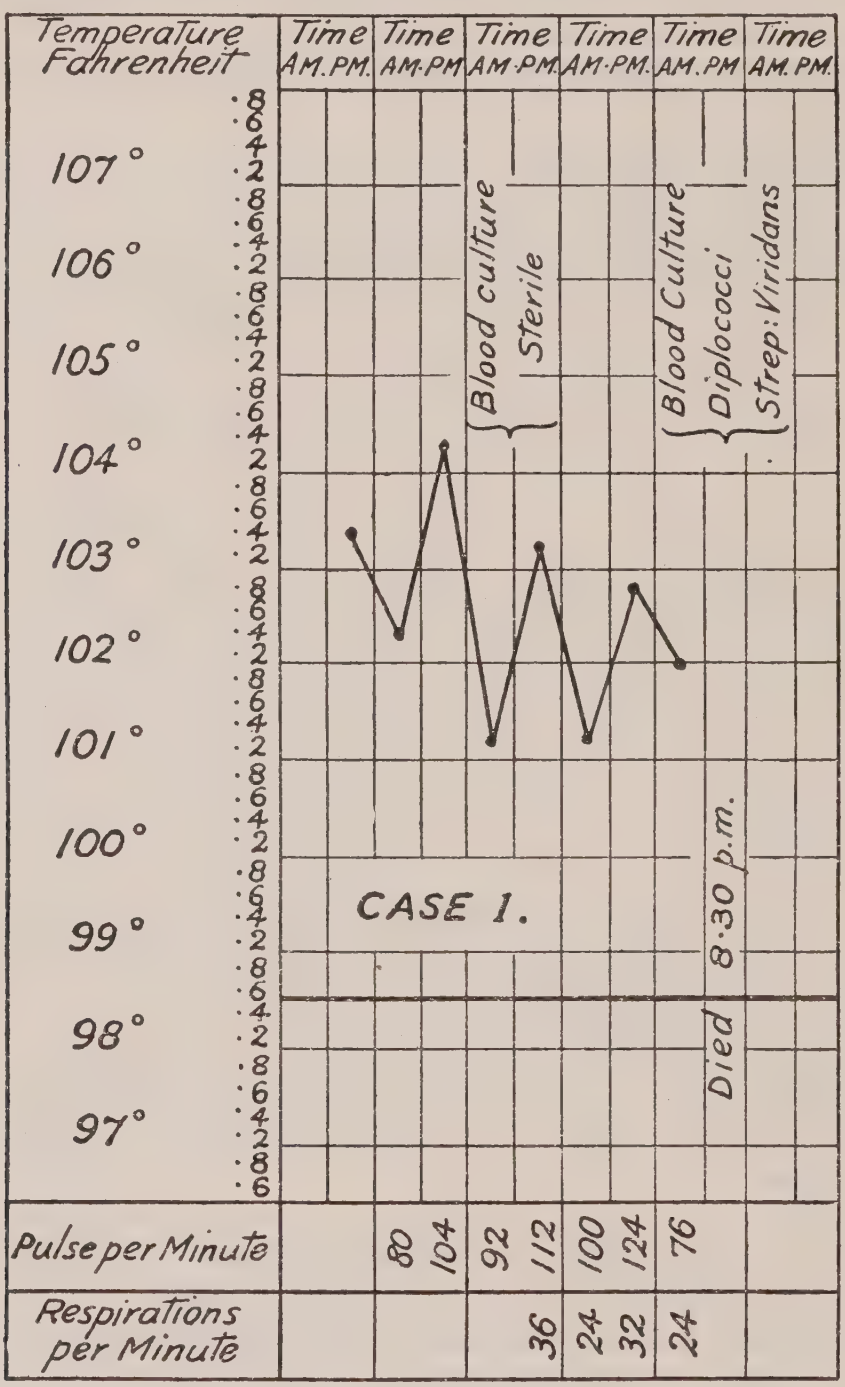
In one case very severe double nerve-deafness persisted for several weeks.

Case Records.

Case I. Ra.

Age 22. Onset Oct. 23. Admitted to hospital Oct. 25. Died Oct. 28 (6th day).

Oct. 25. Cyanosed. Rhonchi and râles over both lower lobes. T. 102°–104·2°. P. 104–90. Blood culture sterile. Throat swab—*B. influenzae, S. viridans.*



Oct. 27. Extension of signs to right upper lobe. T. 101·2°–102·8°. P. 100–124. R. 24–32.

Oct. 28. Moribund. Blood culture, diplococci and *S. viridans.*

Post mortem.

Epiglottis. Inflamed.

Aryteno epiglottidean folds. Oedematous.

Larynx. Intensely inflamed.

Trachea. Intensely inflamed.

Pleura. ℥x turbid semipurulent fluid in left sac. Haemorrhages on diaphragmatic surface of visceral pleura and anterior surface of lungs.

Right lung. Lower lobe and part of middle lobe red, homogeneous, gela-

tinous. Tortoise-shell appearance of surface. Upper lobe solid, granular, pus in bronchi.

Left lung. Upper lobe aerated. Lower lobe pouring fluid on section.

Liver. Large, pale, greasy patches on surface and in substance.

Spleen. Normal.

Suprarenals. Normal.

Kidneys. Normal.

Cultures post mortem.

Lung. *B. influenzae* and *S. viridans.*

Bronchial fluid. *B. influenzae* and *S. viridans.*

Heart blood. *S. viridans.*

Pericardial fluid. *S. viridans.*

Special Features. A case fatal on 6th day, presenting high, falling temperature, relatively slow but quickening pulse, little respiratory distress, great involvement of lung.

Microscopic appearance.

Right lung. Lower lobe. Section shows intense congestion. The alveoli are filled with fibrinous exudate, which shows a varying amount of leucocytic invasion. Around the smaller bronchi leucocytic accumulation is marked.

Upper lobe. Shows great congestion. The alveoli contain a granular deposit with very few leucocytes.

Case II. Mu.

Age 27. Onset Oct. 23. Admitted Oct. 25. Recovered.

On admission flushed, T. 104·6°, P. 100, R. 28. Impaired percussion note and air-entry at both bases. Slight friction right lower axilla.

5th day of illness. Friction ceased. Air entry still impaired. Sputum clear, viscid. T. 102°–101·4°. R. 30.

6th day of illness. Temperature normal. P. 72. R. 20.

7th–11th day. Temperature subnormal. General condition, no distress. Cough persisting. Sputum mucopurulent.

12th day. Relapse pyrexia to 102·4°. P. 92. No distress and no fresh lung-signs.

13th day. Temperature fell to normal during the day and remained subnormal till 18th day.

18th day. Transient rise of temperature to 101°.

Subsequent course : steady convalescence.

Summary. Uncomplicated case. Relative slowness of pulse during course and convalescence. Two pyrexial relapses. Ultimate complete recovery.

Throat swab. 4th day. *B. influenzae* and meningococci (?). No pneumococci or streptococci.

Naso-pharyngeal swab. 4th day. *B. influenzae,* meningococci (?). No streptococci or pneumococci.

Case III. Pe.

Age 27. Onset Oct. 22. Admitted Oct. 25. Died 16th day.

On admission (4th day). T. 103·2°. P. 100. R. 42. Signs of bronchitis general, but especially evident in right lower and left upper lobes. Sputum frothy. No signs of consolidation. Throat swab, *S. salivarius, S. viridans.*

6th day. Signs more extensive. Sputum more abundant and very watery.

8th day. Increasing respiratory distress. Cyanosis. Temperature falling to 100°. Pulse-rate raised to 116. Sputum blood-stained, *B. influenzae,* a few *S. viridans.*

10th day. Sputum purulent. Coarse crepitations general. Impaired movement of right chest and bronchial breathing over right lower lobe.

10th–16th day. Steady increase in severity of pulmonary symptoms, with extension of broncho-pneumonic patches, falling temperature with rising respiration-rate.

Bacteriology.

Post mortem. Pericardial fluid, negative. Pleuritic exudate, haemolytic streptococci and *S. viridans.* Fluid from bronchi, *B. influenzae* in large numbers and numerous *S. viridans.* Tracheal fluid, a few colonies of haemolytic streptococci.

Sputum. B. influenzae (profuse growth), *S. viridans, M. catarrhalis.*

Naso-pharyngeal swab. S. viridans, S. salivarius.

Post mortem.

Pericardial effusion. 6 ounces clear fluid (sterile).

Heart. Thin walled and flabby.

Larynx. Inflamed.

Trachea. Much inflamed; containing milky, frothy fluid.

Pleura. Gelatinous lymph on surface of right lower lobe.

Right lung. Surface haemorrhages. Haemorrhagic infarct (?) in upper lobe. Appearance of grey hepatisation in greater part of lower lobe and small part of middle lobe.

Left lung. Surface haemorrhages. Upper lobe engorged and pouring fluid on section. Lower lobe capillary broncho-pneumonia with multiple 'pock-mark' abscesses.

Summary of Case. Fatal case with lung complications present on the 4th day. Lung condition increasing in extent and severity. Haemorrhagic process well marked.

Microscopic appearance.

Left lower lobe. Shows abscess formation with complete solution of bronchial wall and cavitation due to formation of pus. Great engorgement of whole section; remaining alveoli are filled with fibrin and leucocytes with a varying admixture of red blood corpuscles.

Case IV. Wu.

Age 34. Onset Oct. 22. Admitted Oct. 24. Complete recovery.

On admission. T. 103. P. 90. R. 24.

3rd day. Weak breath sounds and few crepitations at left base only.

4th day. T. 100·8°–102·4°. Air entry good at both bases. Few crepitations on inspiration R. and L. Sputum scanty, viscid, white.

Naso-pharyngeal swab. B. influenzae. Gram-negative diplococci, probably meningococci. No growth on plain agar.

5th day. Lung signs clearing. Temperature normal. R. 20. P. 70.

7th day. No signs in lungs. No rise of temperature.

Summary. An uncomplicated case, rapid fall of temperature. No relapse.

Case V. Wa.

Age 19. Onset Oct. 21. Admitted Oct. 25. Recovery.

On admission (5th day). Fauces injected. Lumbar pain. T. 103·6°. P. 104. R. 44. Substernal pain. Inspiratory crepitations right upper lobe and upper part of left lower lobe. Pink sputum.

6th day. Impaired air entry and percussion-note both lungs. Many crepitations. No bronchial breathing.

Naso-pharyngeal swab. Pneumococci, *B. influenzae*, haemolytic streptococcus.

8th day. Definite tubular breathing at apex of right lower lobe. Impaired note and many moist sounds at both bases. Sputum muco-purulent.

10th day. No tubular breathing. Coarse crepitations both lower lobes. Fine crepitations both upper lobes. Sputum muco-purulent.

12th day. Signs clearing.

15th day. Aphonia.

19th day. Voice returned, convalescence established.

Clinical Summary. Severe case in young subject with pulmonary complications. Rapid respiration and early haemoptysis. Relatively slow pulse through out. No cyanosis. Steady convalescence and complete recovery.

Case VI. Gr.

Age 38. Onset Oct. 26. Admitted Oct. 28. Recovery.

On admission. Much cough, scanty sputum.

3rd day. Moist sounds at left base, air entry poor. T. 103°. P. 108. R. 26.

Naso-pharyngeal swab. B. influenzae, staphylococci, and a gram-negative diplococcus, *M. catarrhalis.*

5th day. Little sputum, becoming muco-purulent. More moist sounds in chest. Air-entry good.

7th day. Temperature normal in morning. Practically no chest signs. Cough loose and decreasing.

9th day. Convalescence established.

Clinical Summary. A mild uncomplicated case ending in complete recovery.

Case VII. We.

Age 32. Onset Oct. 30. Admitted Oct. 30. Recovery.

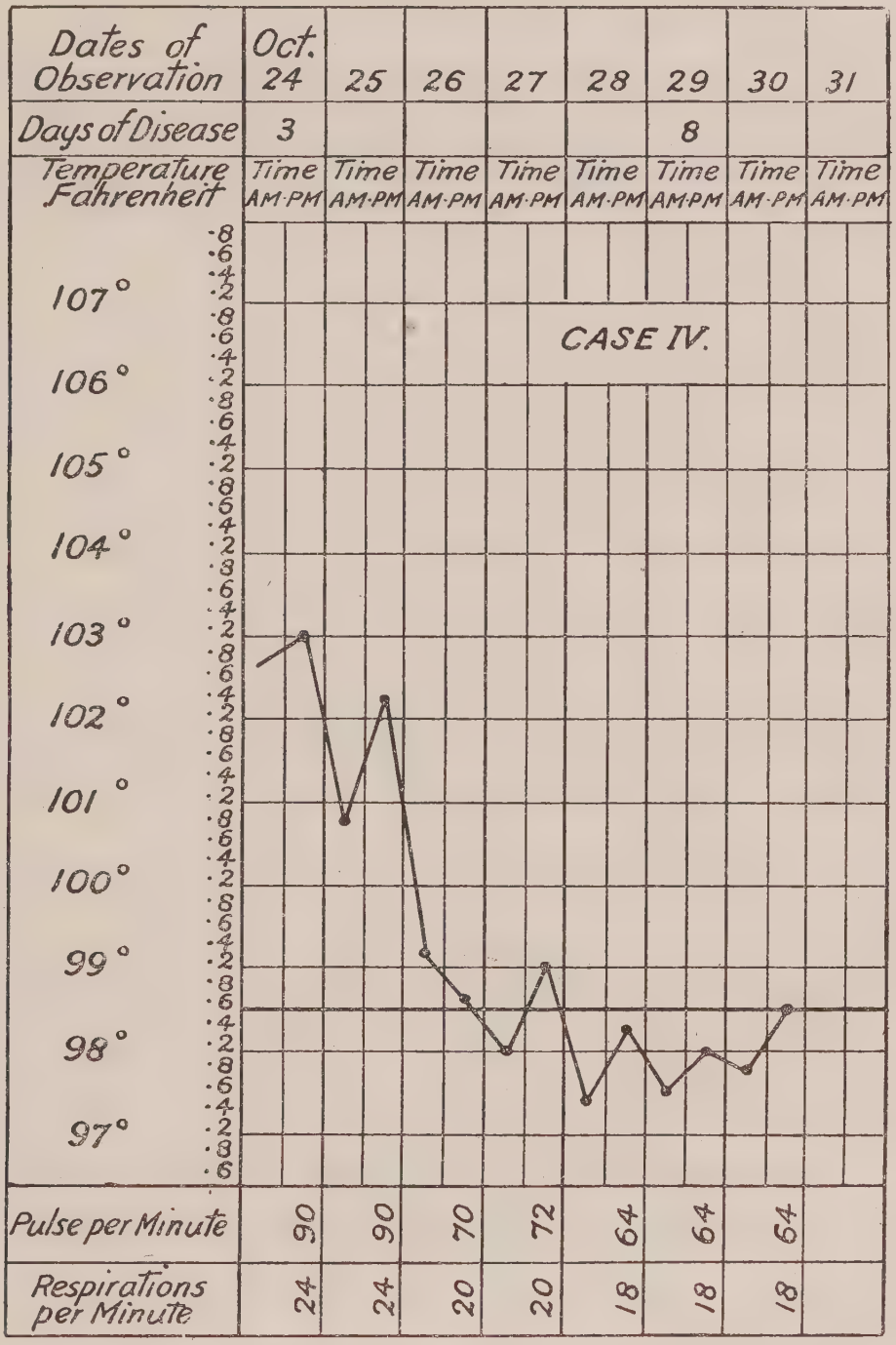
Onset sudden with shivering, faintness, pains all over. Slight dry cough. No chest signs.

Naso-pharyngeal swab. *B. influenzae*, Vincent's organisms, *S. viridans.*

3rd day. Still no sputum. Severe pains in muscles of thigh, and legs.

5th day. Relapse pyrexia. No chest signs.

Summary. A mild uncomplicated case seen from time of onset. Early relapse. Complete recovery.



Case VIII. Kl.

Age 21. Onset Oct. 31. Admitted Nov. 1. Recovery.

On admission. T. 104°. P. 72. R. 24.

2nd day. Scanty sputum. Coarse crepitations both bases, especially left.

Naso-pharyngeal swab. Haemolytic streptococcus. *B. influenzae.*

Sputum. *M. catarrhalis* and *S. viridans.*

4th day. Definite bronchial breathing left upper lobe. Many moist sounds left lower and right lower lobe. T. 104·2°. P. 72. R. 24.

6th day. Temperature falling. No bronchial breathing.

8th day. Temperature normal. Lung signs clearing.

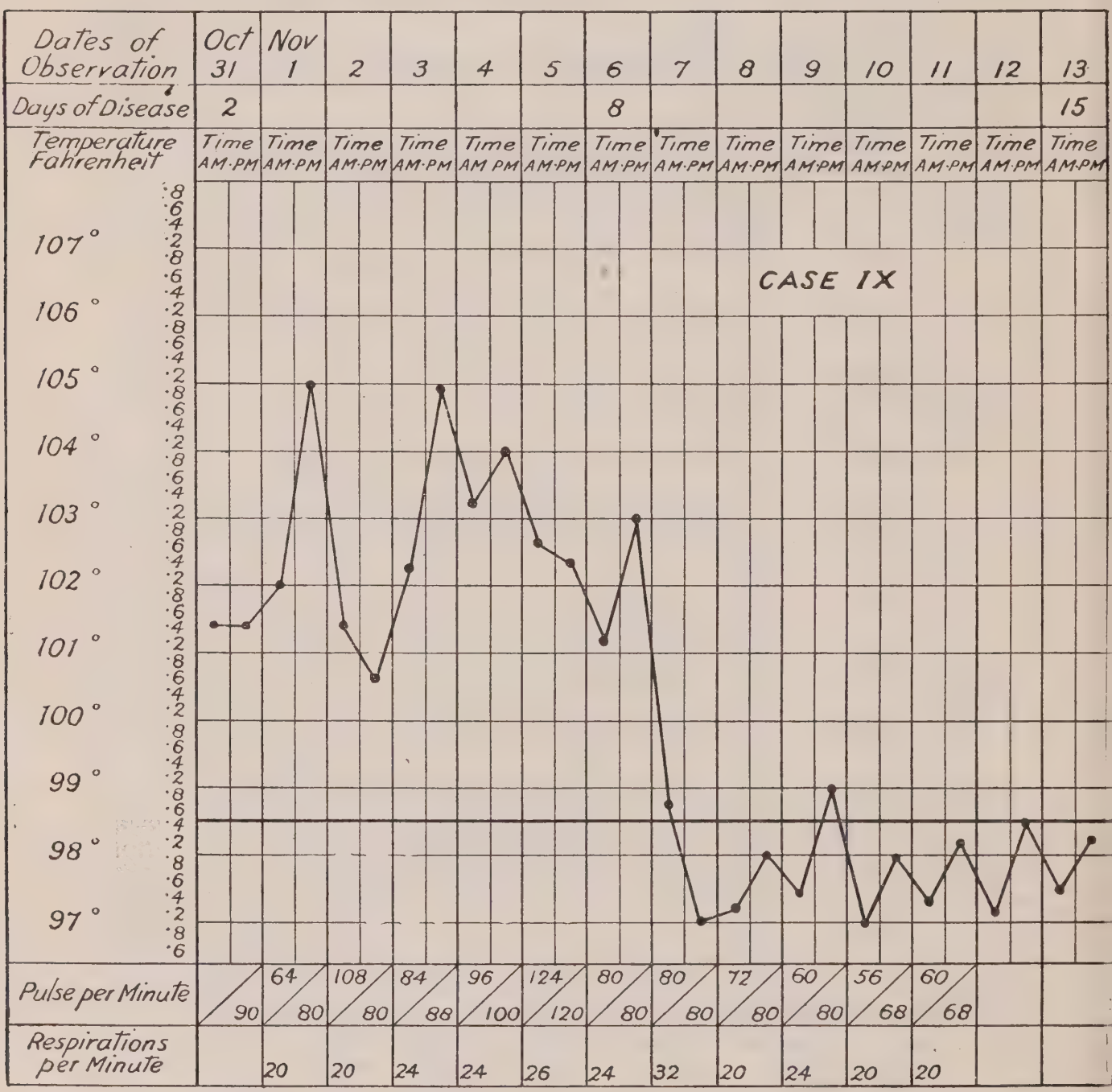
Clinical Summary. A case of moderate severity with definite lung complications. Pulse and respiration slow throughout. Rapid improvement of lung condition. Steady convalescence.

CASE IX. Ri.

Age 20. Onset Oct. 29. Admitted Oct. 31. Recovery.

Onset, sudden with shivering, headache, cough, and limb-pains.

3rd day. T. 105°. P. 80. R. 20. Coarse crepitations left base. Sputum viscid, white.



Throat swab. B. influenzae. M. catarrhalis, a few pneumococci (?).

Sputum. B. influenzae, a very few Diplococci.

5th day. T. 105°. P. 88. R. 24. Crepitations in both lungs. No tubular breathing. Sputum more liquid.

7th day. Delirious (mild). Sputum purulent (no blood-staining). Temperature falling. Pulse rising.

8th day. Mentally clear. P. 80. R. 24. Temperature falling.

10th day. Temperature subnormal. Few moist sounds only in lungs.

Clinical Summary. Severe case with mild pulmonary involvement. Remarkable absence of pulse or respiration disturbance. Complete recovery.

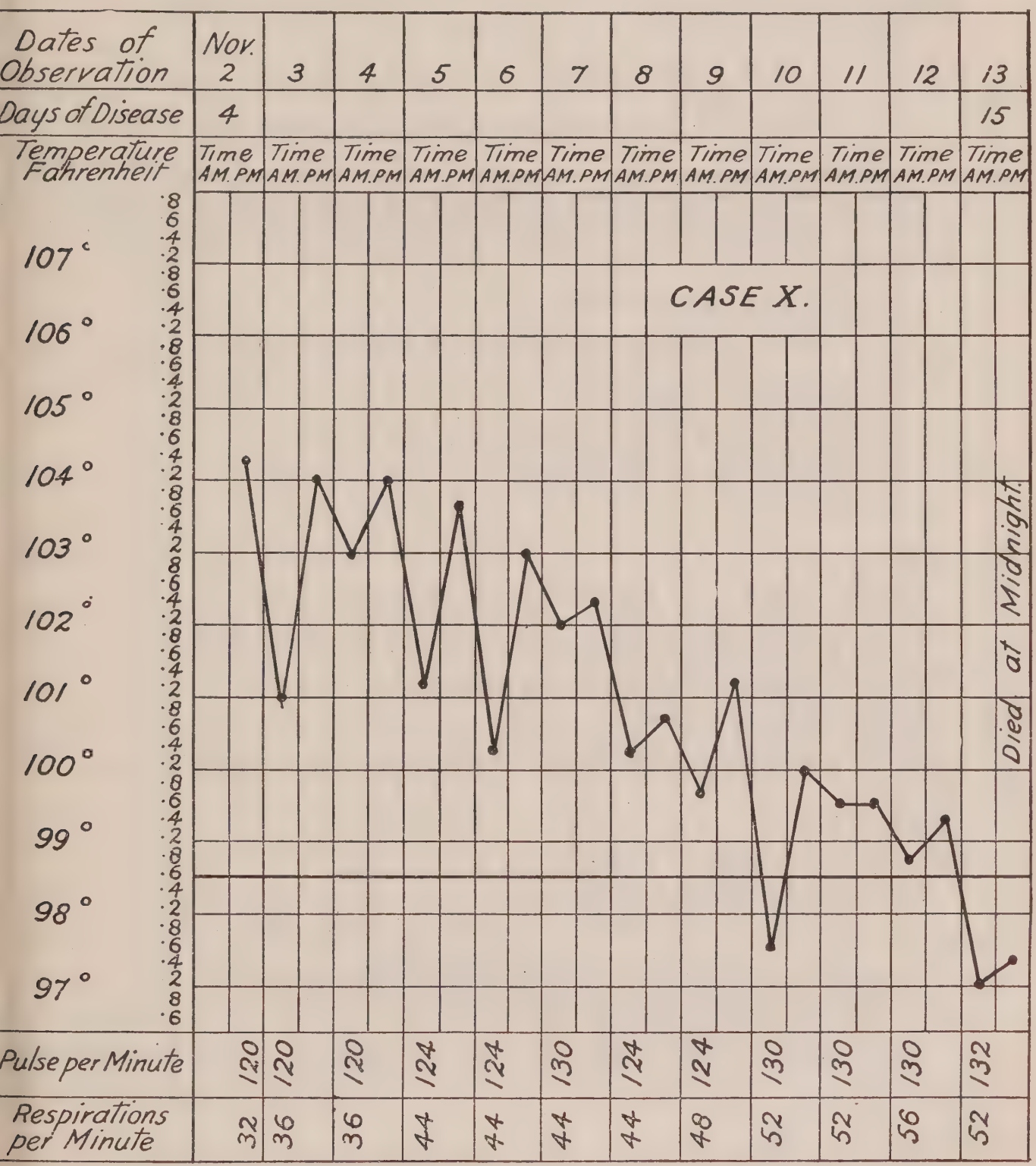
Case X. Be.

Age 23. Onset Oct. 30. Admitted Nov. 2. Died 16th day.

Onset with rigor, cough, and general pains.

5th day. Cyanosed, delirious. Consolidation right upper lobe. Crepitations and poor air-entry over left lower lobe. T. 104·2°. P. 120. R. 32.

7th day. No change.



9th day. Consolidation left lower lobe. No change in right lung.

11th day. More distressed. Temperature falling. Respiration rising.

13th day. More distressed. Cough ceased.

16th day. Died.

Post mortem.

Pharynx. Retronasal abscess.

Trachea. Mild inflammation.

Tracheal glands. Enlarged.

Pericardium. 10 oz. of clear effusion.
Heart. Normal.
Pleura. Surface haemorrhages, fibrin on lung surfaces.
Right lung. Upper lobe, grey, streaming fluid on section, breaking down in parts, pus in bronchioles. Middle lobe clear. Lower lobe some surface haemorrhages.
Left lung. Upper lobe, greyish pink, mottled, granular, moist. Lower lobe, patchy consolidation.
Liver. Moderately fatty, much engorged.
Spleen. Normal.
Kidneys. Pale, swelling of cortex.
Suprarenals. Healthy.
Clinical Summary. A fatal case in a young subject, early severe involvement of lung.
Microscopic Appearance.
Left lower lobe. Section shows almost universal consolidation with intense engorgement and destruction of bronchial mucous membrane in smaller bronchi. Leucocytic infiltration of fibrinous exudate is present through the whole section. No abscess formation.
Pleura. Shows fibrinous pleurisy.
Bacteriology (post mortem).
From retronasal abscess. *B. influenzae* in large numbers and a few *S. viridans.*
Pericardial fluid. Negative.
Heart's blood. Negative.
Bronchial fluid. *S. viridans* and *B. influenzae.*
Tracheal scraping. *B. influenzae* only.
Lung culture. An occasional diplococcus.
Pleural pus.

Case XI. Ho.

Age 32. Onset Nov. 1. Admitted Nov. 2. Recovery.
Onset sudden. General pains.
3rd day. Cough, little frothy sputum. T. 101·4°. P. 84. R. 20. Few scattered moist sounds in chest.
4th day. Sputum. *B. influenzae* and *S. viridans.*
5th day. Chest signs clearing.
8th day. Temperature normal. Chest clear.
Clinical Summary. A very mild uncomplicated case with complete recovery. No relapse.

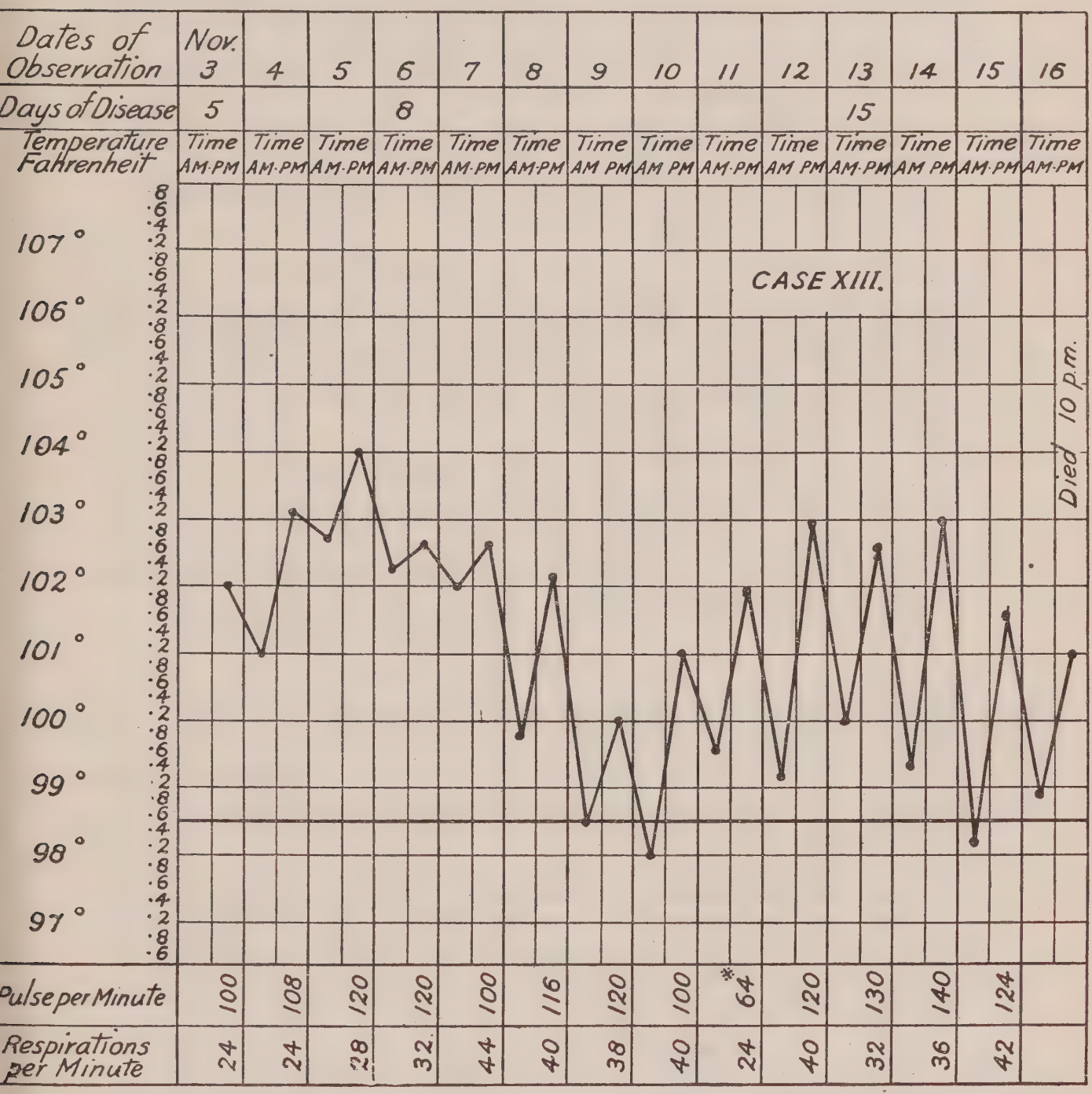
Case XII. Bu.

Age 28. Onset Nov. 3. Admitted Nov. 3. Recovery.
Onset with shivering, general limb-pains.
On admission. T. 103°. P. 80. Dry cough: scanty viscid sputum. No signs in chest.
2nd day. T. 100°. P. 60. R. 20. No chest signs.
Sputum. B. influenzae, M. catarrhalis, and *S. viridans.*
3rd day. Temperature normal. No discomfort.
5th day. Relapse pyrexia: shin pains.
Clinical Summary. A very mild case of influenza or more probably a case of trench fever.

Case XIII. Me.

Age 27. Onset Oct. 30. Admitted Nov. 3. Died 18th day.
On admission (5th day). T. 102°. P. 100. R. 24. Substernal pain. No distress. Crepitations right and left base and right submammary. Sputum scanty, viscid.
7th day. Moist sounds general.
8th day. Respiration hurried at times.
10th day. Much distressed. Sputum abundant, frothy.
12th day. Blood in sputum: creamy pus.
13th day. Chest full of moist sounds, dullness both lower lobes.

15th day. Cyanosis and great distress.
16th day. Cough ceased.
18th day. Died.
Post moriem.
Larynx. Moderate inflammation.
Trachea. Moderate inflammation. Creamy pus in trachea, no froth, no blood.
Pericardium. 10 oz. of fluid.



Heart. Dilatation of right chambers.
Pleura. Right obliterated by old adhesions. Left, 1–2 oz. of cloudy fluid.
Right Lung. Studded throughout with small patches of broncho-pneumonia. Small abscess cavity in lower lobe.
Left Lung. Upper lobe aerated, but pouring fluid on section. Lower lobe many patches of broncho-pneumonia.
Liver. Fatty.
Spleen. Small and soft.
Clinical Summary. A fatal case with early pulmonary complications. Transient improvement followed by extension of involvement of lung.

Microscopic Appearance.

Left lower lobe. Section 1 shows universal consolidation and great engorgement with definite small haemorrhages into alveoli scattered through section. Leucocytic invasion of fibrinous exudate, though apparent through section, is more marked around the smaller bronchi, some of which show destruction of mucous lining.

Section 2 shows patchy consolidation in which the intensity of the injection is concentrated round the smaller bronchi. In some mucous membrane has been destroyed, and in others both mucous membrane and fibrous wall are in the process of destruction. Leucocytic accumulation, marked at these points, diminishes as one passes to the parenchyma of lung, and, finally, on the fringe of a bronchopneumonic area, alveoli are found containing fibrin only.

Bacteriology.

Heart's blood. Negative.
Pericardial fluid. Negative.
Bronchial fluid. *B. influenzae* (numerous), *S. viridans.*
Tracheal scraping. *B. influenzae* (profuse), *S. viridans.*
Pleural exudate. *B. influenzae* (profuse), *S. haemolyticus, S. viridans.*
Lung. *B. influenzae* and *S. viridans.*

CASE XIV. Fa.

Age 26. Onset Nov. 3. Admitted Nov. 3. Recovery. Onset with shivering, headache, substernal pain and aching limbs. No signs in chest. T. 102·4°. P. 80. R. 20.

3rd day. Few moist sounds in chest. Scanty viscid sputum.
6th day. Epistaxis. Cough looser.
7th day. Sputum frothy mucus.
8th day. Epistaxis ceased. Temperature normal.
10th day. Sputum slightly muco-purulent.
12th day. More cough, scattered moist sounds in chest.
14th day. Convalescence established.

Clinical Summary. A case of moderate severity, uncomplicated, ending in complete recovery without relapse.

Sputum. Haemolytic streptococcus, *S. pyogenes*, no *B. influenzae.*

CASE XV. Wh.

Age 20. Onset Nov. 7. Admitted Nov. 8. Died 11th day. Onset sudden with shivering, headache, and vomiting.

2nd day. Sputum frothy, mucoid, white, unstained. Crepitations over left upper lobe. Bases clear. T. 103·8°. P. 100.

3rd day. Vomiting continues. Sputum mucopurulent. Impaired note and air-entry at right base.

5th day. Vomiting continues. Sputum pink, frothy.

7th day. Vomiting continues. Sputum rusty. Consolidation, right lower lobe.

8th day. Fall of temperature. Greater distress. Abundant liquid stained sputum. Vomiting ceased.

10th day. *In extremis.* Irregular pulse.

11th day. Died.

Post Mortem.

Larynx. Not inflamed.
Trachea. Slightly inflamed, containing brown frothy fluid.
Tracheal Glands. Swollen.
Pleura. Recent dry pleurisy over left lower lobe.
Right Lung. Deep area of consolidation in upper lobe. Middle and lower lobes not involved.
Left Lung. Massive consolidated areas in lower and part of upper lobes.
Pericardium and Heart. Normal.
Liver. Diffuse and localized pallor in left lobe.
Spleen. Soft and swollen.
Kidney. Swelling of cortex, with blurring of structure.

Clinical Summary. A fatal case in a young subject with obstinate vomiting, some haemoptysis, and severe pulmonary complications.

Microscopic Appearance.

Left lower lobe. Section shows great engorgement and almost universal consolidation. Small scattered areas of haemorrhage are present. Leucocytic invasion of consolidated area is universal, being more marked at the sites of the smaller bronchi.

High power. Shows cellular accumulation to consist of (1) polymorphonuclear leucocytes; (2) mono-nuclear cells (small), probably young filioblasts and lymphocytes; (3) mono-nuclear cells (large), probably catarrhal cells derived from cells lining alveoli.

Bacteriology.

Heart's blood. Negative.

Pericardial fluid. Negative.

Bronchial fluid. *B. influenzae* and *S. viridans.*

Tracheal Scraping. Gram-negative diplococci do not grow on plain agar, probably meningococci.

Pleural exudate. *B. influenzae*, a few staphylococci.

Lung. *S. viridans.*

Tracheal fluid. Negative.

Case XVI. Pe.

Age 31 years. Onset Nov. 11. Admitted Nov. 13. Recovery. Onset with substernal pain and cough, backache and limb-pains. No shivering.

3rd day. T. 102. R. 22. P. 84. Many crepitations at left apex (this patient had previously been under observation as a case of suspected tuberculosis). Scattered rhonchi. Sputum white and frothy.

5th day. T. 100. P. 80. R. 24. Chest signs unchanged. Sputum mucopurulent.

6th day. T. normal. More moist sounds in chest.

8th day. Chest signs clearing.

10th day. Convalescence established.

12th day. No relapse. Still a few signs at left apex.

Clinical Summary. A mild case in a subject of slight chronic bronchitis, no complications, complete recovery without relapse.

Naso-pharyngeal swab (17.11.18). *B. influenzae, S. viridans.*

Case XVII. Gl.

Age 19. Onset Nov. 12. Admitted Nov. 16. Died 11th day.

On admission (5th day). T. 104·4°. P. 106. R. 40. Crepitations and impaired air-entry over upper part of right lower lobe. Sputum viscid, pink.

6th day. Left lower lobe solid. Many crepitations, left lower lobe. Sputum more fluid.

7th day. Delirious. Cyanozed. No sputum.

8th day. Great distress. T. falling. Cough ineffectual. Consolidation of left lower lobe.

9th day. Subcutaneous emphysema of neck.

11th day. Died.

Post mortem.

Subcutaneous emphysema of face, neck, and trunk.

Larynx. Acutely inflamed.

Trachea. Intensely inflamed with haemorrhagic areas and necrosis of mucosa.

Pleural sacs. Dry. Haemorrhages on lung-surfaces.

Right lung. Capillary broncho-pneumonia involving larger portion of all lobes.

Left lung. Upper lobe emphysematous. Lower lobe, massive bronchopneumonia.

Heart and Pericardium. Normal.

Liver. Many fatty areas.

Spleen. Soft.

Kidneys. Cortex pale, swollen, structure blurred.

Suprarenals. Healthy.

Clinical Summary. A fatal case in a young subject, pulmonary complications arising early. Very intense tracheal inflammation. Extensive subcutaneous emphysema.

Naso-pharyngeal swab (19.11.18). *B. influenzae, S. viridans.*

Histology. Left lung, lower lobe. Section shows almost universal consolidation and great engorgement. Leucocytic penetration and infiltration of the inflammatory exudate is advanced. Bronchi in section are filled with leucocytes and desquamated epithelial cells. Destruction of the ciliated bronchial mucous membrane is marked. There is no abscess formation. Great destruction of interalveolar septa is present, section being almost unrecognizable as lung tissue.

Case XVIII. Ca.

Age 22. Onset Nov. 22. Admitted Nov. 22. Recovery. Onset with substernal pain, shivering. T. 102·4°. P. 112. R. 20.

1st day. Air-entry unimpaired. Few crepitations left base. Dry cough, viscid sputum.

2nd day. T. falling. Signs unaltered.

4th day. T. 99·6. More cough, sputum scanty, mucopurulent. No chest signs.

6th day. T. normal.

8th day. Relapse. T. 101·4°. P. 104. R. 38. No fresh signs in chest.

10th day. T. subnormal. No complaints. Cough practically ceased.

Clinical Summary. Mild uncomplicated case in young subject. One relapse followed by complete recovery.

Naso-pharyngeal swab (23.11.18). *B. influenzae, S. viridans.*

Case XIX. Ca.

Age 28. Onset Nov. 22. Admitted Nov. 24. Recovery.

On admission, T. 104. P. 84. R. 20.

3rd day. Dry cough, very scanty sputum. Substernal pain. No signs in chest.

5th day. T. 102°. Cough looser. Few moist signs at left base.

7th day. T. 99°. Chest clear.

8th day. T. normal. Cough much less.

Clinical Summary. Mild uncomplicated case. Complete recovery without relapse.

Bacteriology. No examination.

Case XX. Pr.

Age 39. Onset Nov. 29. Admitted Nov. 29. Recovery.

1st day. Shivering, general pains, headache, vomiting. Crepitations and prolonged expiratory murmur at both bases. Scanty viscid sputum.

3rd day. Vomiting ceased. Pain in limbs persists. Lung condition unaltered. Urine contains faint trace of albumen, no cells, no casts. T. 101·4°. P. 90. R. 24.

5th day. T. normal. No pain. Urine normal. Few moist sounds at bases only. Cough ' loose '.

7th day. Evening T. 99°. No complaint.

9th day. Convalescence established.

Clinical Summary. A mild short uncomplicated case in a middle-aged subject.

Bacteriology. No examination.

3 (*b*). THE MORBID ANATOMY OF INFLUENZA

BY

MAJOR C. E. SUNDELL, R.A.M.C.

THE following account is based upon the findings on post-mortem examination of sixty-two cases.

I. EXTERNAL APPEARANCES.

Wasting has not been a striking feature.

Rash was present in two cases : it was profuse, petechial, macular : the face was not affected.

Subcutaneous emphysema was extensive in one case.

Oedema was not seen.

II. MUSCLES.

Interstitial haemorrhage into the rectus abdominis had occurred in two cases.

III. RESPIRATORY SYSTEM.

A. *Pharynx.*

Congestion was common, purple coloration was occasionally seen.

Retronasal abscess was present in one case.

B. *Larynx.*

Congestion was common, sometimes intense with purple discoloration.

Aryteno-epiglottidean folds were often oedematous.

C. *Trachea.*

Inflammation found in every case, often confined to region below fourth tracheal ring, sometimes intense : haemorrhagic areas and shallow necrosis of the mucosa seen occasionally.

Tracheal contents usually frothy fluid, often blood-stained.

Tracheal glands swollen, reddish purple, never suppurating.

Bronchial glands often swollen and engorged.

D. *Pleural Sacs.*

Frequently containing moderate effusion, clear with a little floating lymph. Haemothorax never seen.

Pleural surfaces : superficial haemorrhages were very common especially on the posterior surfaces of the lungs. Thin fibrinous pleurisy often seen over involved areas of the lung but strikingly less than that found over areas of pneumonic consolidation.

E. *Mediastinum.*

Emphysema of upper and anterior mediastinum was seen in three cases.

F. *Lungs.*

Very striking changes were found :

(*a*) ' Oedema ' ; (*b*) ' gelatinization ' ; (*c*) haemorrhage ; (*d*) broncho-pneumonia ; (*e*) abscess formation was seen in three cases which had died at an early stage.

(a) ' *Oedema ' of lung.* Distribution roughly lobar. The affected area is firm, heavy, crepitant, retains its shape, has a smooth surface often showing petechial haemorrhages : on section no consolidation is present ; the cut surface pours out fluid which is clear, slightly brownish and frothy. No pus can be squeezed from the bronchioles.

This type of lung occurs in early cases who have shown much frothy liquid sputum during life and in whom the affected area has given a flat note on percussion, air entry has been poor and many inspiratory and expiratory crepitations have been present.

(b) ' *Gelatinization ' of lung.* Distribution roughly lobar, both lungs usually involved, right middle lobe often free. Surface is smooth or thinly coated with fibrin. On section the lung is red, homogeneous, glassy ; it contains excess of fluid and is nearly airless.

The physical signs presented during life are similar to those of the ' oedema ' cases but the dullness is more marked, air entry is more impaired, and occasionally tubular breathing is audible.

(c) *Haemorrhage.* Haemorrhage into the lung substances has been common. The haemorrhagic areas are irregular in size and shape ; sometimes they are conical suggesting infarction. The condition is often associated with broncho-pneumonia, from which it cannot be differentiated during life.

(d) *Broncho-pneumonia* and capillary bronchitis are common in all grades in late cases. ' Miliary broncho-pneumonia ' was seen in four cases.

IV. Circulatory System.

A. *Pericardium.*

Moderate effusion ($\bar{z}$x) was not rare. It was sometimes cloudy but never blood-stained.

Petechiae of the visceral and parietal surfaces were very common.

B. *Heart.*

Nothing abnormal was detected except flabbiness of heart-muscle and usually dilatation of r. chambers.

Valves and aorta were always normal.

V. Alimentary System.

A. *Liver.*

Pallor was very common. It was usually concentrated in patches on and near the surface, especially of the left lobe, but was often diffuse.

Associated with this in a few cases was a slight fibrosis.

B. *Spleen.*

The only abnormality detected was occasionally slight swelling. The organ was usually soft and engorged.

C. *Pancreas.*

Normal.

D. *Intestine.*

Slight injection of mucosa of colon was occasionally seen. No haemorrhagic areas were found. Intestinal lymphatic glands were normal.

E. *Peritoneum.*

Normal.

VI. Other Organs.

A. *Kidneys.*

Little change was noted other than that associated with a febrile illness. In a few cases marked pallor and swelling of the cortex with blurring of the structure suggested subacute nephritis.

B. *Suprarenals.*

No haemorrhages were seen in cortex or medulla.

C. *Brain.*

No abnormality was discovered on naked-eye examination of the brain or meninges.

3 (*c*). THE MORBID HISTOLOGY OF INFLUENZA

BY

Captain J. I. Connor, A.A.M.C.

The material for this study was obtained from post-mortems on cases of influenza conducted by Major Sundell, R.A.M.C., whose description of the gross morbid anatomy appears elsewhere in this monograph.

Sections of tissue were fixed in 10 per cent. formalin solution, and the usual routine for paraffin sections was followed. As a general rule haematoxylin and eosin were used for staining, but variations were made where necessary for special examinations, i. e. fat, bacteria, capsules, &c.

I. Lungs.

The histological examination of the lungs being of great importance is divided into five headings, as follows :

A. Areas of haemorrhage.
B. Areas of broncho-pneumonia.
C. Areas of oedematous pneumonia.
D. Areas of grey hepatization with multiple abscess formation.
E. Areas of consolidated lung ranging in colour from red to grey and usually 'dripping'.

A. *Areas of Haemorrhage.* (See Plates I and XI A).

The predominating factor is haemorrhage into the alveoli. Great engorgement of all the vessels is present. The majority of the alveoli are plugged with inflammatory exudate, many showing the reticulum of fibrin with a varying admixture of red blood corpuscles. In some areas these red blood corpuscles have undergone haemolysis and in others still retain their original shape. Occasionally a group of alveoli is seen filled with blood only and in many places rupture or solution of the inter-alveolar septa is apparent. The picture is one of acute haemorrhagic inflammation with toxic spoiling of the capillaries. Although cellular reaction is not marked at this stage, it is most evident in and around the bronchioles, many of which are filled with polymorphonuclear leucocytes and débris of desquamated ciliated epithelial cells. In some cases the mucous membrane is totally destroyed, leaving the fibrous walls exposed and even split by the cellular infiltration, with partial solution. The sub-bronchial lymphatic spaces are also infiltrated by leucocytes. Passing to the parenchyma of the lung, leucocytes (largely polymorphonuclears) are dotted sparingly through the mixture of haemorrhage and inflammatory exudate. Many large phagocytic mononuclear endothelial cells can be seen, some still attached to the alveolar walls, and some forming part of the imflammatory exudate filling the alveoli.

B. *Areas of Typical Broncho-pneumonia.* (See Plate XI B).

Under the low power patchy areas of inflammatory change are apparent and the infection is concentrated around the smaller bronchi. Stages in the process of their destruction can be seen.

(1) Desquamation of the mucous membrane with leucocytic accumulation in the lumen.

(2) Infiltration of the sub-mucous and fibrous layer.

(3) Gradual solution and splitting of the fibrous tissue of the wall.

The nucleii of ciliated cells are swollen and granular, and the surrounding alveoli, where evident, are filled with inflammatory cells. In many cases the small area of lung affected, and appearing as a white nodule at autopsy, is unrecognizable as lung tissue, consisting only of a mass of leucocytes, fibrin, catarrhal cells, and remnants of destroyed lung tissue.

Passing to the parenchyma of the lung, the alveoli are seen retaining their shape, and filled with inflammatory cells and fibrin; alveoli with fibrinous reticulum, or homogeneous albuminous exudate only, and finally empty alveoli somewhat distorted during fixation, with slight inflammatory change. The picture is one of patchy inflammation concentrated round the bronchioles with marked cellular reaction, and destruction of tissue but with little haemorrhage. Sections stained by Giemsa stain show that bacteria are most numerous in the leucocytic plug in the bronchioles and neighbouring tissues, becoming less frequent as the parenchyma is approached.

C. *Areas of Oedematous Pneumonia.* (See Plate XII A).

Lung section. There is no universal consolidation and destruction of tissue is not marked. Section on examination appears as an open network of interalveolar septa except those parts approximating the bronchioles. General engorgement is marked and groups of alveoli throughout the section are filled with red corpuscles. The bronchioles show marked damage with desquamation of the ciliated epithelium and partial solution of the fibrous wall. Leucocytic reaction is marked in and around these bronchioles, many being filled with a leucocytic plug. Approximate alveoli are also plugged with a mixture of inflammatory cells.

Passing to the parenchyma of the lung, capillary engorgement is marked. The phagocytic cells lining the alveoli are markedly active, showing increase in size and a tendency to become detached and to accumulate in the alveoli.

The majority of the alveoli contain only a few free endothelial cells, leucocytes, and granular débris, these alveoli being filled at autopsy with oedema fluid.

D. *Areas of Grey Hepatization and Abscess Formation.* (See Plate XII B).

Almost universal consolidation is present and cellular reaction is marked. Every alveolus, still recognizable as such, is filled with phagocytic macrophages from the alveolar walls, and smaller mononuclear cells. This cellular accumulation is marked in certain areas, identified as sites of smaller bronchi, by remnants of ciliated epithe-

lium and fibrous tissue of the wall. Tissue destruction in these areas is marked, and multiple abscess cavities are present. The surrounding lung tissue is not recognizable as such, being converted into a mass of inflammatory cells. The parenchyma of the lung shows some alveoli (usually grouped) filled with red blood corpuscles.

A fibrinous pleurisy is commonly seen with early organization of the fibrin.

E. *Areas of consolidated Lung of varying Colour.* (See Plate XIII).

The three sections already described are definite types seen during the epidemic, and every transition may be seen from one to the other, and a combination of all three in the same lung.

As a rule the acute cases, where early death has supervened, show a markedly haemorrhagic lung. Where death occurred later in the infection the usual change of colour, apparent in lobar pneumonia, is present. Grey hepatization, purulent infiltration, or multiple abscess formation alter the picture and always show a patchy distribution, in contradistinction to lobar pneumonia where an entire lobe or the whole of one or both lungs have suffered equally.

Sections stained for bacteria by Giemsa (Pasteur Institute), twenty-four hours' immersion, show the preponderance of diplococci in our sections, a pleomorphic diplo-streptococcus being very frequent (see Plates VI and VII).

A few bacilli suggestive of *B. influenzae* were seen, but not in quantity as reported by Muir (*B. M. J.*, January 4, 1919). Universal distribution of bacteria is often present; but when scanty the concentration is most obvious in the leucocytic plug filling the bronchioles, some being intra- and some extra-cellular.

The report of Sewell, Newcastle (*B. M. J.*, January 11, 1919), confirms our opinion of the origin and activities of the large mononuclear cell found in alveoli. Our sections show no evidence of hyperplasia of bronchial epithelium, but evidence of destruction is abundant. (Muir, ibid.)

II. Trachea.

Shows acute inflammatory changes with desquamation of the mucous membrane and leucocytic accumulation in the sub-mucous layer.

III. Bronchial Glands.

Show great engorgement and oedema. Vessels are shown in section distended with red blood corpuscles, and individual cells of gland tissue are separated by oedema, giving a clear appearance to the section. No abscess formation or necrosis is to be seen.

IV. Liver.

Shows varying grades of cloudy swelling, and a granular deposit of fat may be demonstrated by special staining methods and is probably due to diminished functional activity of the liver prior to death. Two cases of the series only show fatty accumulation within the liver cells, and in both of these the liver is somewhat cirrhotic. Terminal engorgment is the rule with marked dilatation of the central lobular vein and of the capillaries draining into it.

V. Kidney.

In the milder cases some degree of cloudy swelling is usually present with some terminal engorgement.

Many cases present severe microscopic damage with swelling of epithelial cells of tubules and desquamation, the tubules being filled with granular and epithelial deposit. Great engorgement of capillaries of Bowman's capsule with haemorrhage into the capsule and elsewhere throughout the kidney tissue from toxic spoiling is present. Leucocytic reaction is scanty, the damage being mainly toxic.

VI. Brain.

Apart from some capillary engorgement nothing abnormal is seen.

VII. Rectus Abdominis Muscle (Haemorrhagic).

Section shows haemorrhage spreading along the areolar tissue separating muscle bundles, with some dilatation of small vessels and capillaries. Individual muscle fibres in transverse section show some difference in taking the counter-stain (eosin), some having a violet tinge. The nuclei are definite and no swelling of muscle protein is apparent.

Leucocytic reaction is absent.

Description of Plates I–IV.

Plate I. Lung from a case of Influenza, showing large more or less circumscribed areas of haemorrhage with marked emphysema in other parts.

Plate II. Larynx and trachea from a case of Influenza, showing oedema of the glottis and acute inflammation of the trachea with an overlying membranous exudate.

Plate III. A. Section of monkey lung (Monkey 2) which had been inoculated with filtered sputum, showing haemorrhagic condition. B. Ditto, showing acute congestion with more marked reaction around two calcified areas.

Plate IV. Left lung and trachea of Monkey 2, which had been inoculated with filtered sputum, showing large haemorrhagic area in lung and marked infection of the trachea.

(The descriptions of the figures included in Plates V–XIII are printed on the plates.)

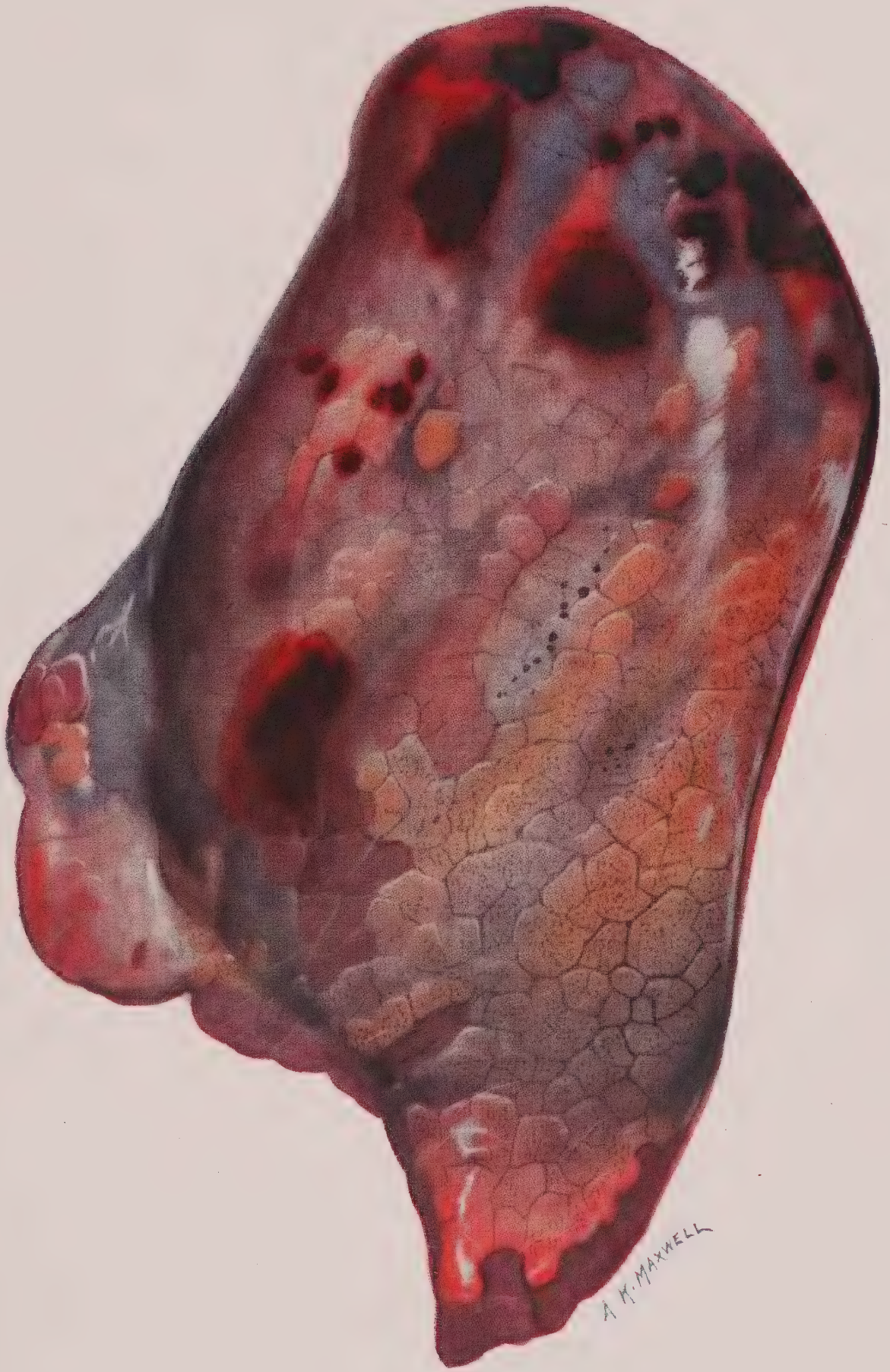

A. M. MAXWELL

Plate II.

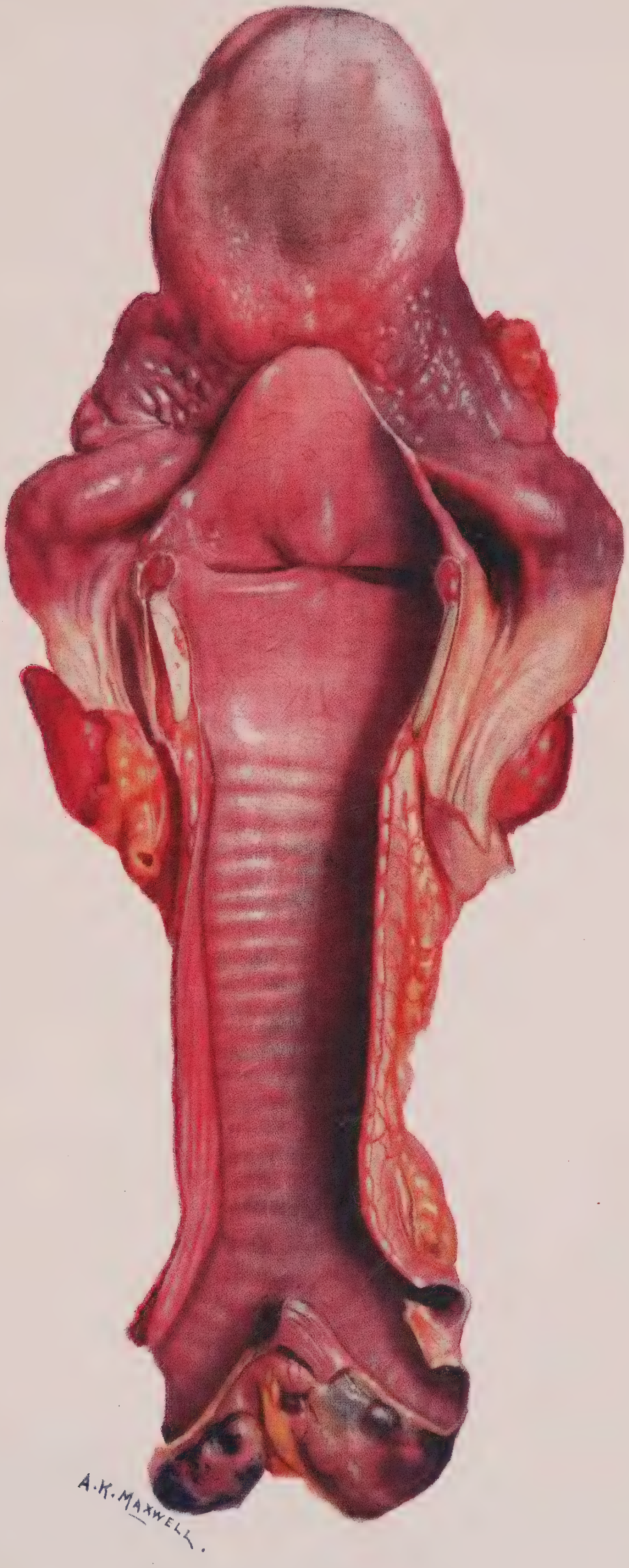


Plate III.

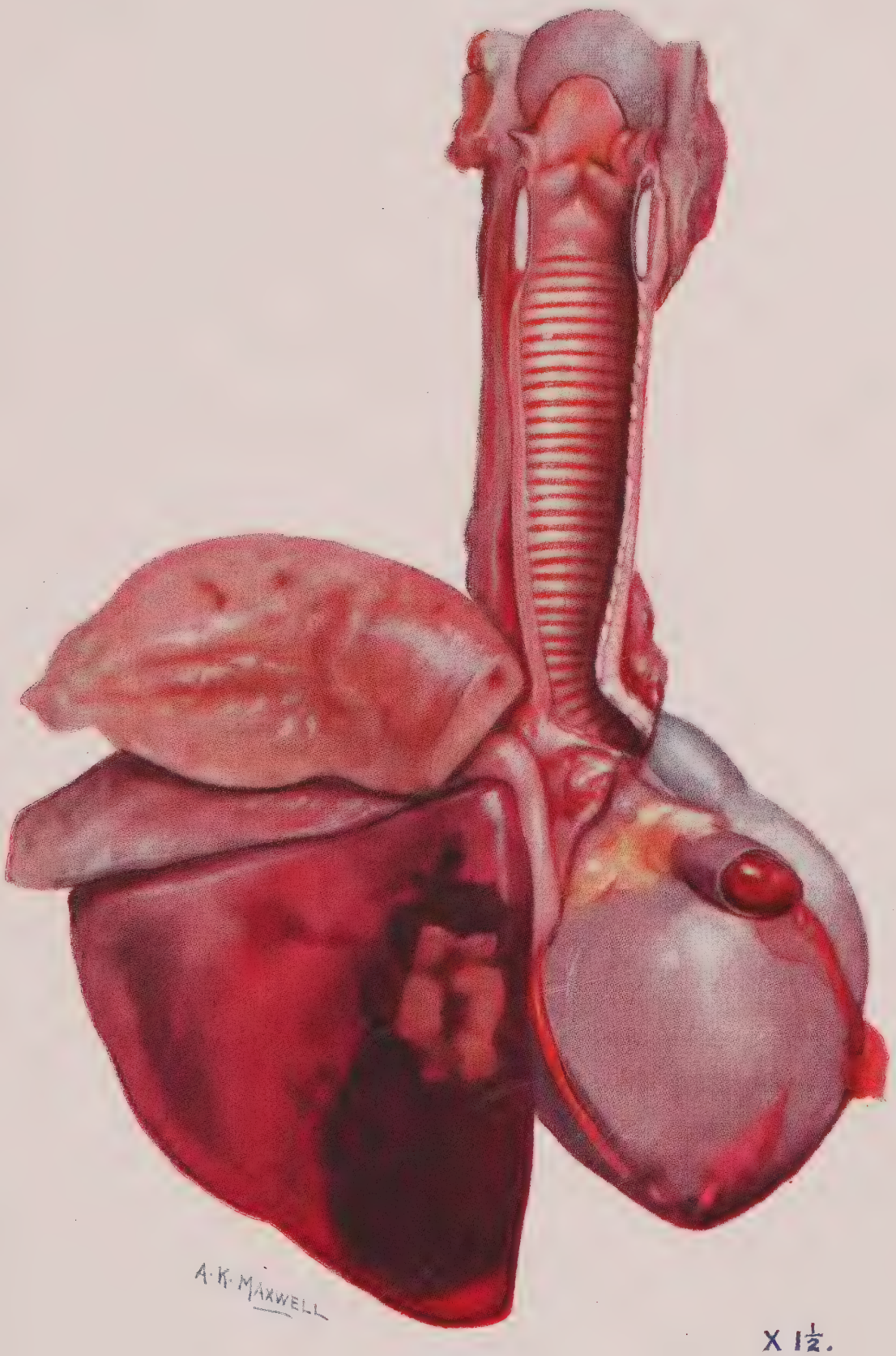
X 2.

A

X 2.

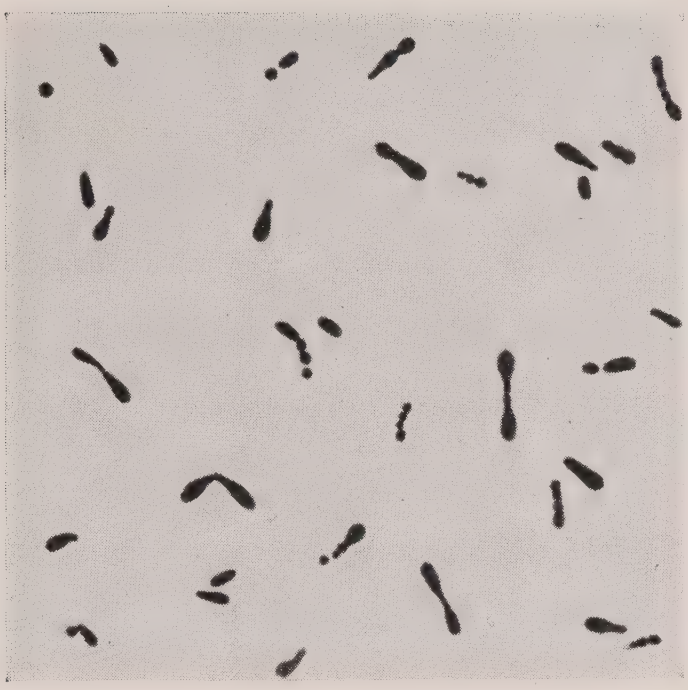
B

late IV.



X 1½.

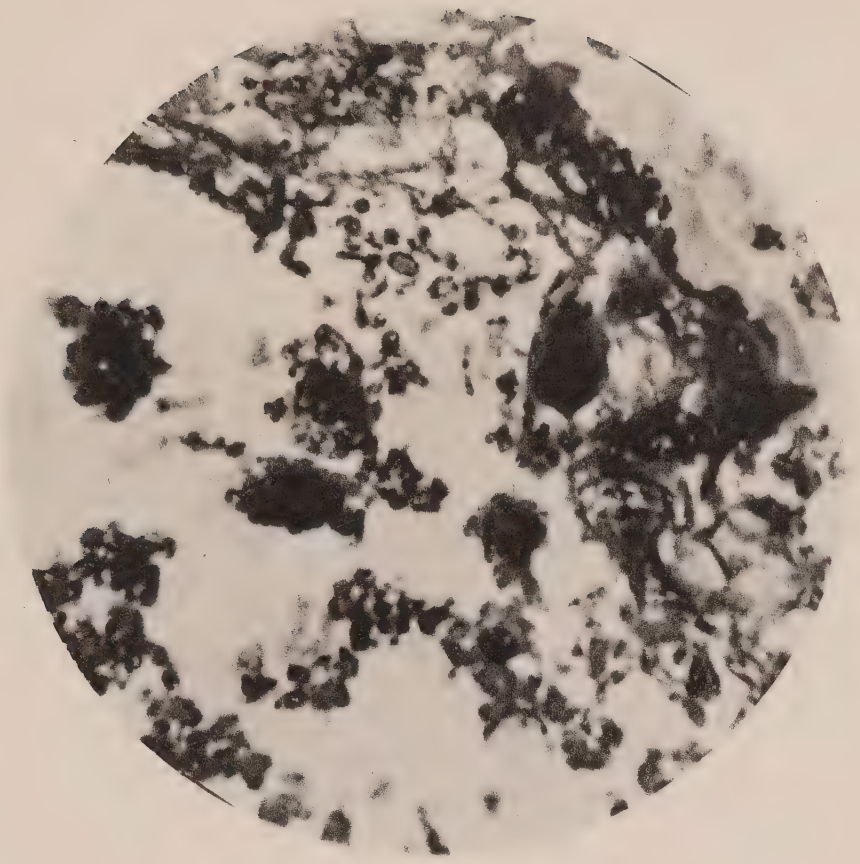
Plate V



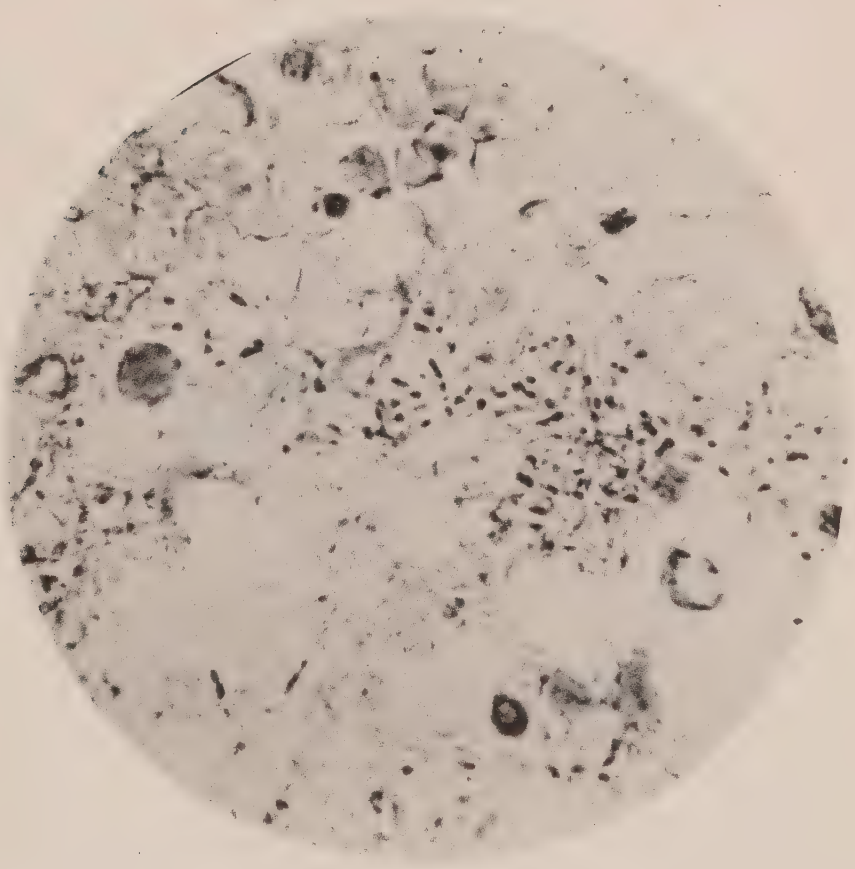
A. A Pleomorphic Diplostreptococcus isolated from a large number of cases of Influenza. From 48-hour culture (× 1500).

B. Diplostreptococcus in association with *B. influenzae*, from 24-hour culture from sputum (× 1000).

Plate VI.

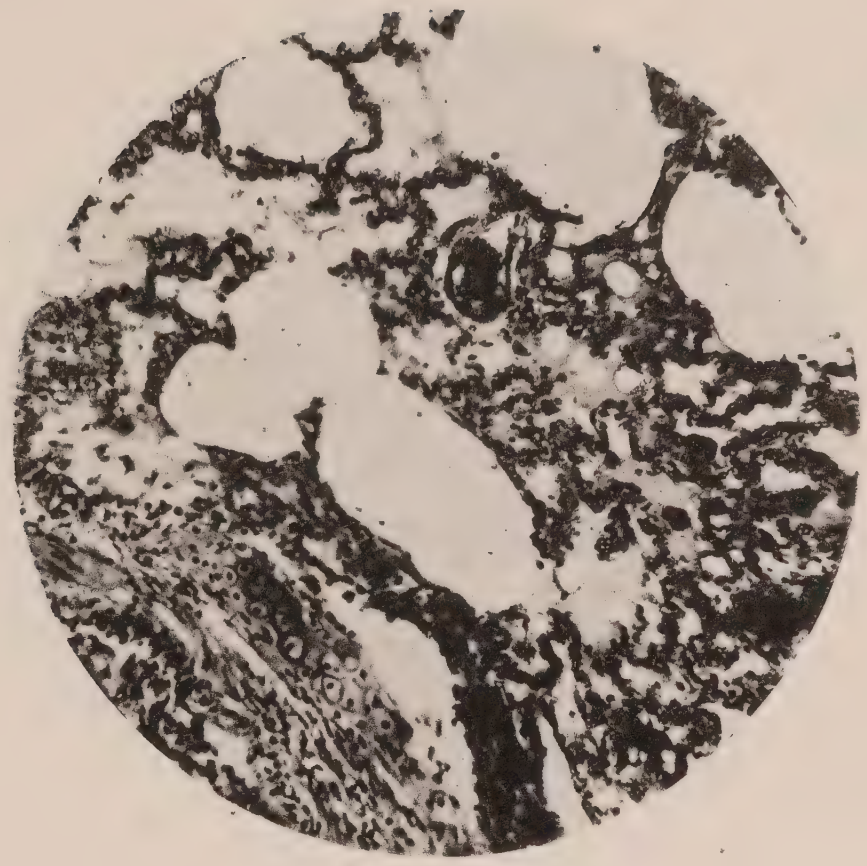


A.—Human Lung, encapsuled diplococci in section (×1500).

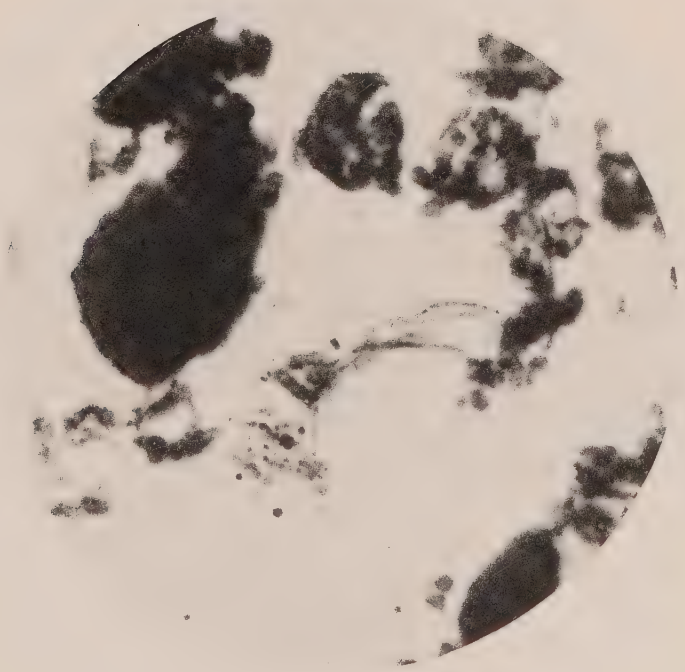


B.—Human Lung, pleomorphic diplostreptococci in inflammatory exudate (×1000).

Plate VII.

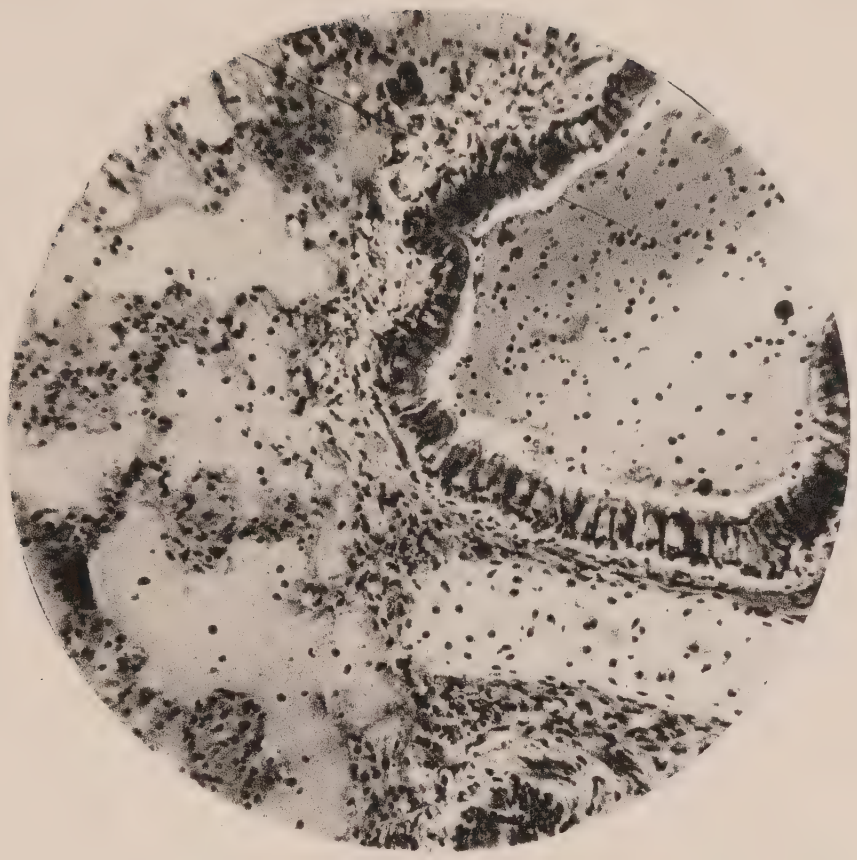


A.—Monkey Lung (Group II, No. 2) showing congestion and inflammatory exudate (×700).

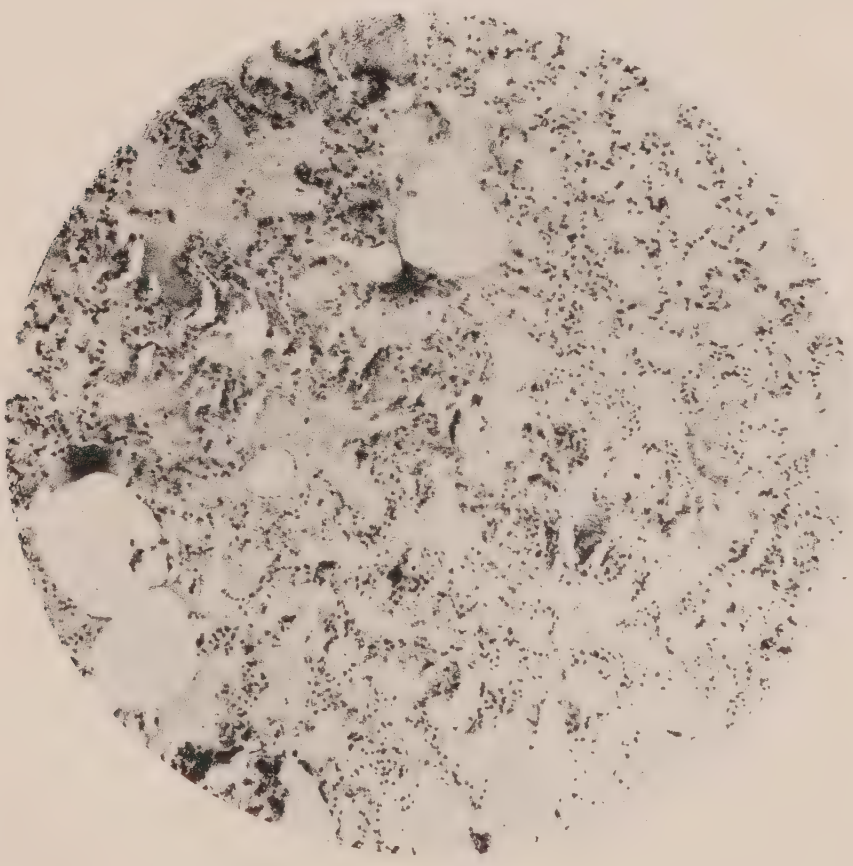


B.—Human Lung showing streptococci in section (×1000).

Plate VIII.

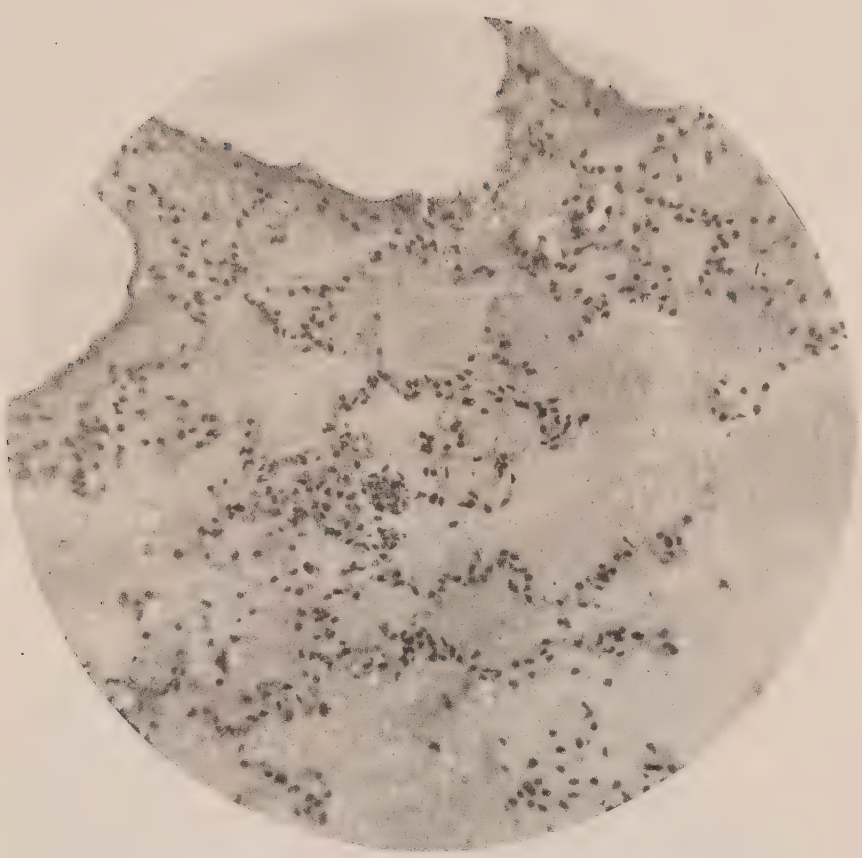


A.—Monkey Lung (Group I, No. **4**) showing inflammatory exudate in alveoli and bronchiole (mucous membrane intact) (×700).

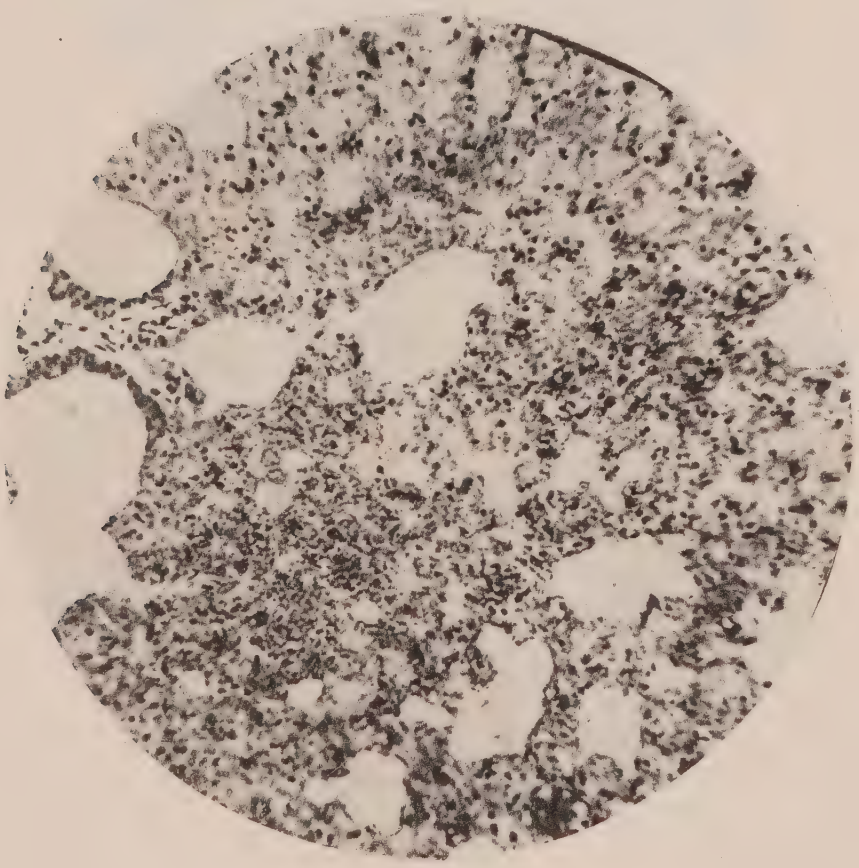


B.—Monkey Lung (Group I, No. **6**) showing margin of inflammatory exudate (left), clear lung (right) (×500).

Plate IX

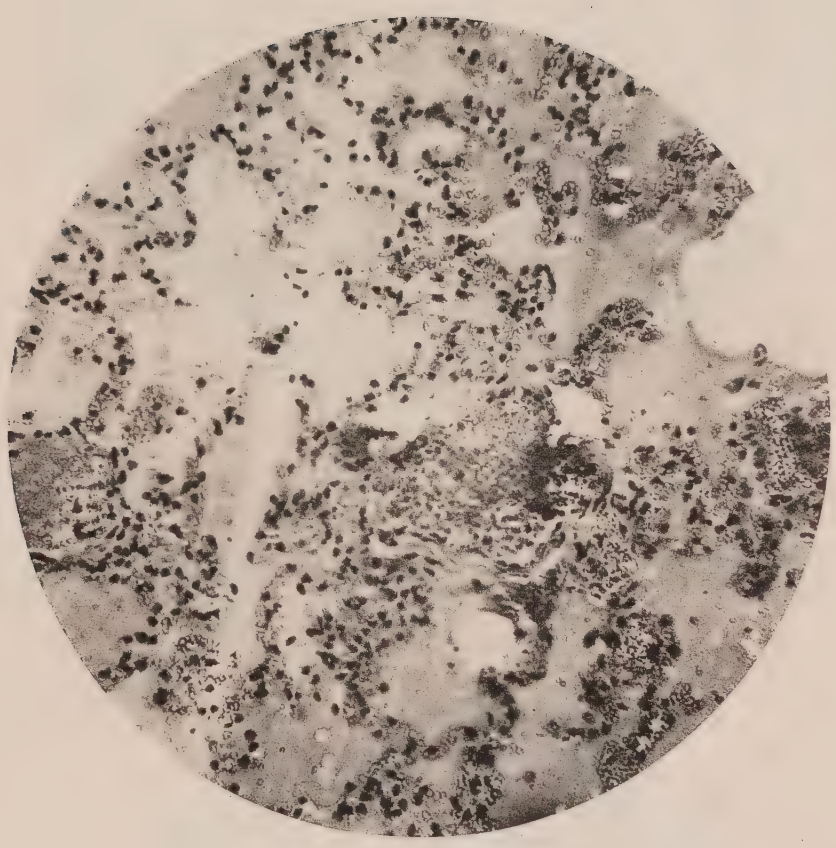


A.—Monkey Lung (Group I, No. 3) showing solution of alveolar wall and inflammatory exudate (×500).



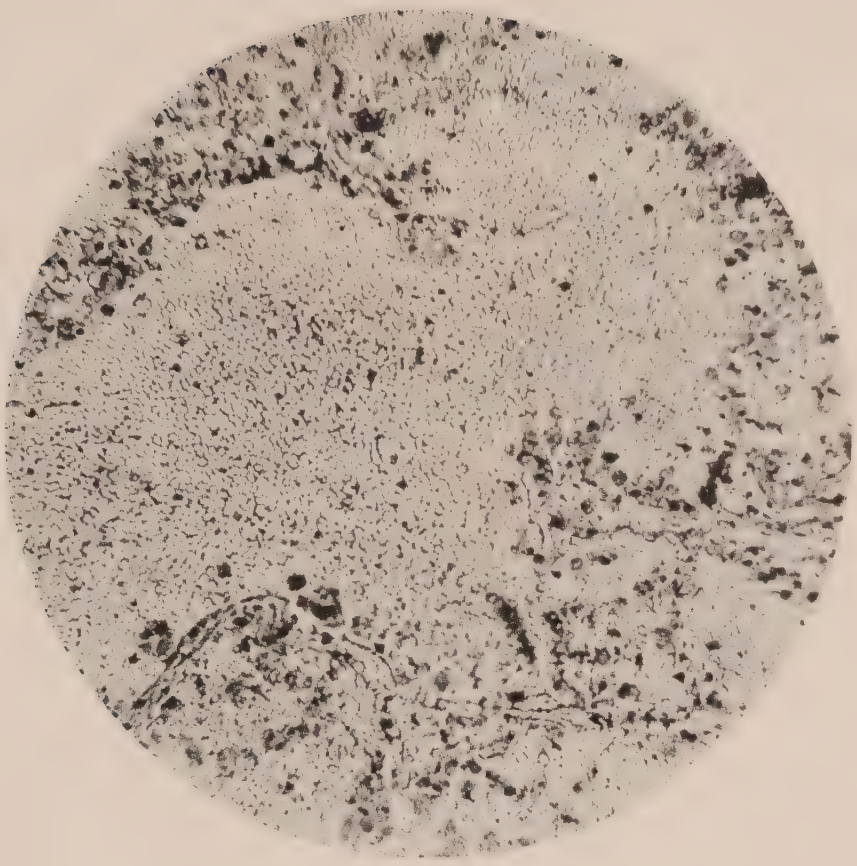
B.—Mouse Lung (W 2) showing haemorrhagic inflammatory exudate (×500).

Plate X.

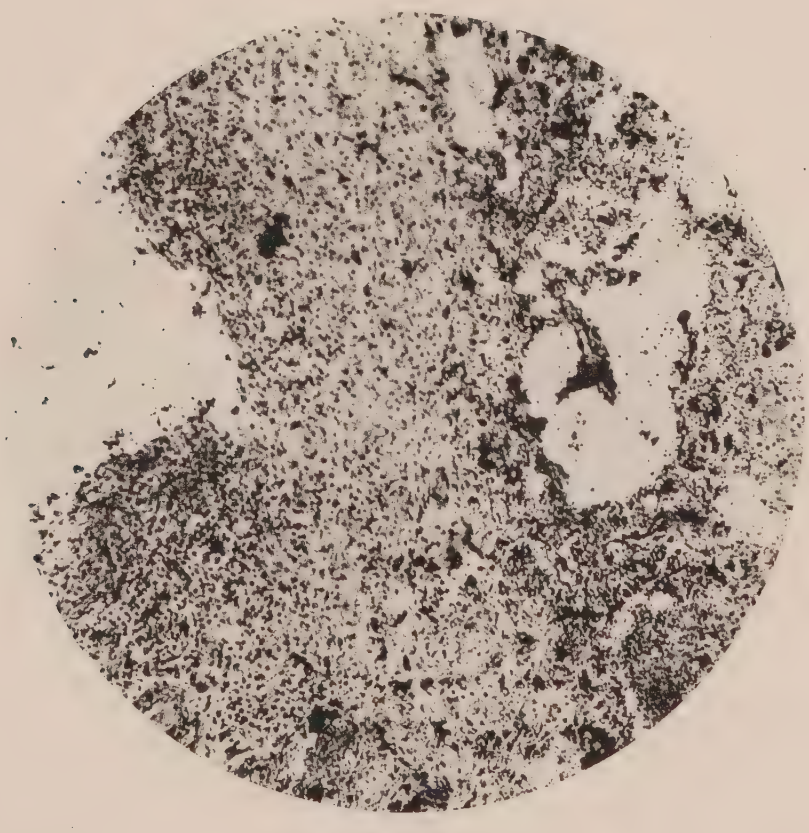


Monkey Lung (Group I, No. 5) showing intense congestion and inflammatory exudate filling alveoli of lung (×500).

Plate XI.

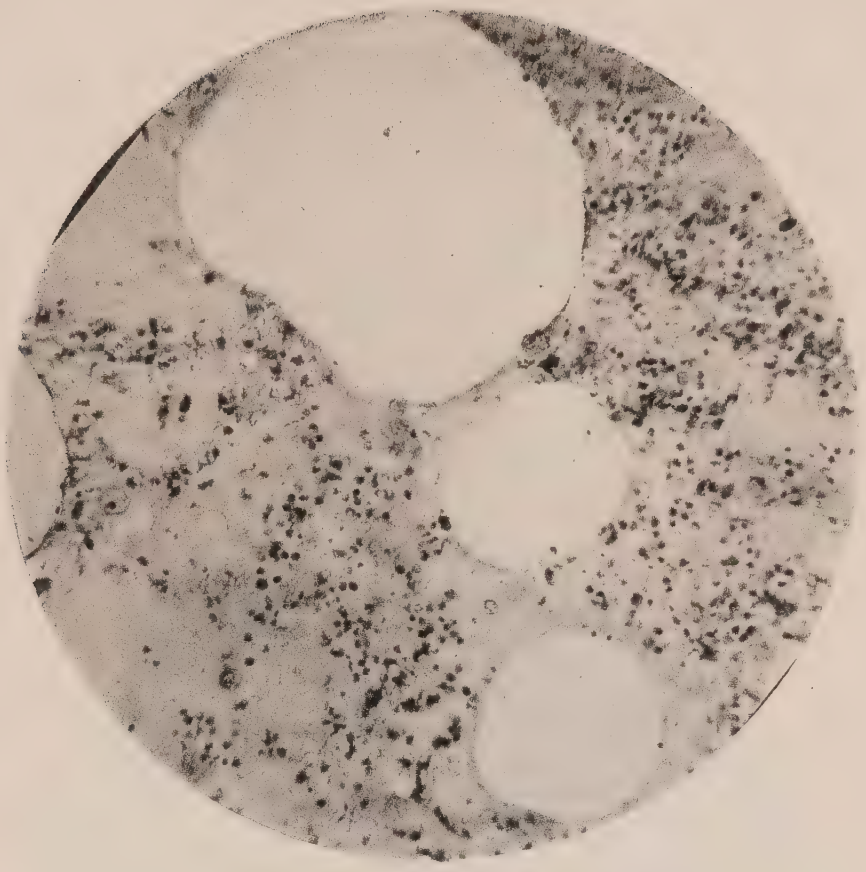


A.—Human Lung. Area of haemorrhage, showing solution and rupture of the alveolar walls and marked accumulation of red blood cells in the surrounding alveoli. Leucocytes scanty ($\times 700$).

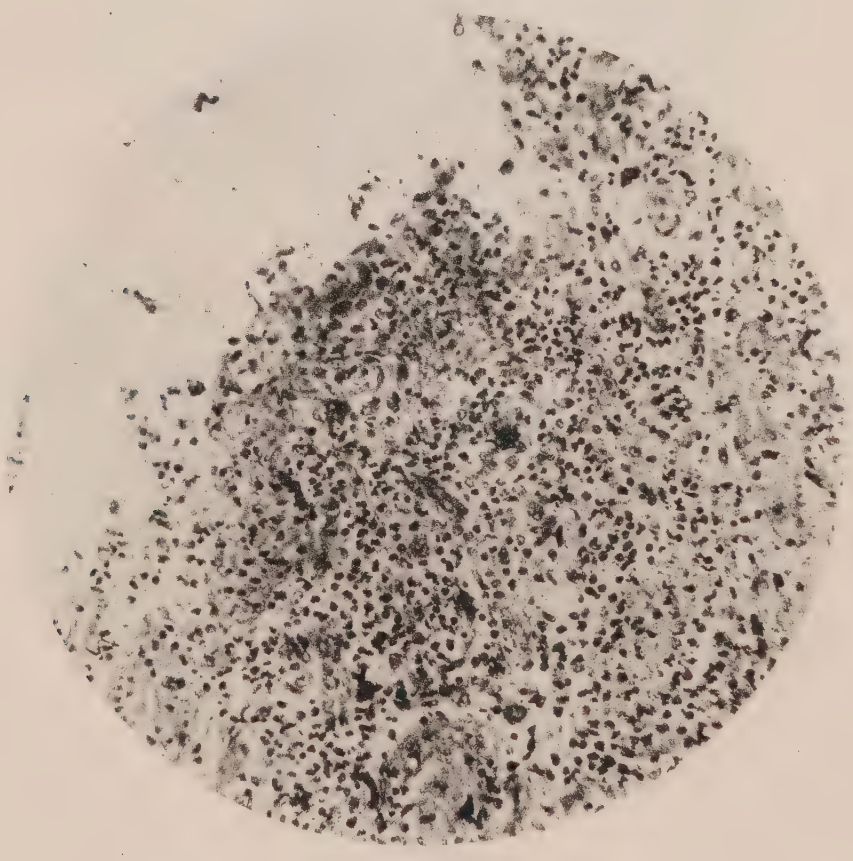


B.—Human Lung. Area of broncho-pneumonia, showing destruction of bronchial mucosa with desquamation of epithelium. Cellular reaction marked at sites of smaller bronchi ($\times 500$).

Plate XII.

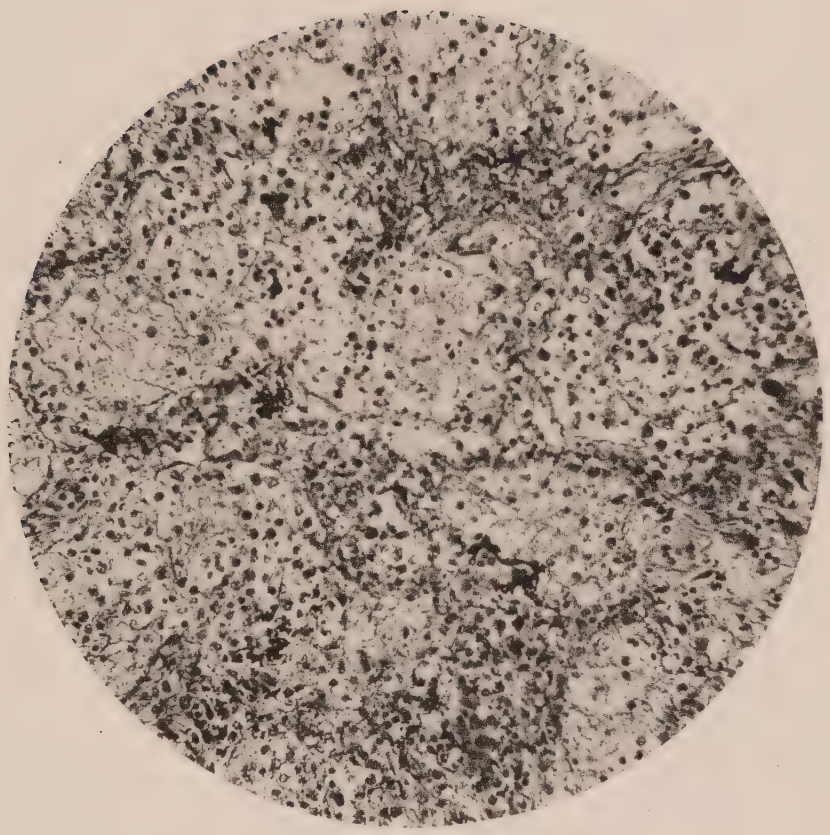


A.—Human Lung, area of oedematous pneumonia, showing alveoli filled with albuminous exudate. Leucocytic reaction not marked. (×700).



B.—Human Lung, area of multiple abscess formation, showing total destruction of bronchial mucous membrane with rupture and solution of the surrounding alveolar walls (×700).

Plate XIII.



Human Lung, area of consolidated lung of varying colour from red to grey, usually dripping at autopsy. Shows a fibrinous reticulum filling the alveoli. Leucocytic reaction present but not advanced ($\times 700$).

PART II

4 (a). CLINICAL IMPRESSIONS OF THE PNEUMONIAS OCCURRING DURING THE INFLUENZA EPIDEMIC

BY

MAJOR (NOW COLONEL) C. F. MARTIN, C.A.M.C.

(From No. 3 Canadian General Hospital.)

THE pneumonias which have occurred during the influenza epidemic of October and November 1918, have been so varied in their clinical characters that it has been impossible to group them into definite types, but the following tentative classification shows the chief types that have been noted :

(1) Cases with symptoms and more or less frank signs of pneumonia, suggesting clinically a lobar pneumonia.

In these cases the sputum may show either *B. influenzae* or organisms of the streptococcus-pneumococcus group as the predominant organism.

(2) Cases in which signs of pneumonia are indefinite and occur only in the later stages of the disease.

(*a*) Mild, or moderately severe cases, with fever lasting from four to eight days, and terminating usually by lysis. The signs of bronchitis predominate.

(*b*) Fulminating cases, fatal in from three to five days, with symptoms suggesting a general septicaemia. The respiratory tract in general shows signs of involvement only in the late stage.

(*c*) Cases with irregular intermittent fever running a severe course for one to four weeks, and terminating fatally or recovering very gradually. In these cases physical examination reveals evidence of an acute involvement of the whole respiratory tract. Signs of pneumonia appear late.

There is frequently epistaxis, pharyngitis, laryngitis, and synchronously a diffuse bronchitis of the finer tubes, and some involvement of the alveoli. Often before death there is evidence of bronchiectasis or abscess formation. The pulmonary involvement is nearly always bilateral, and the physical signs of serious pulmonary disease are usually delayed. To say the least, the ordinary methods of physical examination yield results that are far from satisfactory, and diagnosis from the most careful examination is often quite at variance with the post-mortem findings.

CLINICAL FEATURES OF THE DISEASE.

Onset.

The disease frequently follows more or less exposure, but may come on with unexpected suddenness.

There is usually some malaise, with involvement of the upper respiratory tract, which may develop gradually and progressively.

Chilliness is one of the commonest early symptoms, and may last for days before serious developments occur. A genuine rigor is not so common. Herpes has not been a frequent sign in this epidemic.

Pain.

This has been much less common in the pneumonias of this series than in the ordinary lobar pneumonia with pleurisy.

As a rule, general pains over the body, and especially in the back, predominate over local thoracic symptoms, and the sensation is that of oppression rather than of actual pain.

Cough.

The cough, which is among the earliest symptoms, is at first irritating and non-productive, tracheal in type, and often so continuous as to prevent the patient from getting any rest. Apparently this is one of the frequent causes of the supervening exhaustion.

Marked hoarseness, as a part of the laryngo-tracheitis, has been frequent, and forms a striking feature of the general picture.

Sputum.

In the early stages sputum may be absent for days. It appears first as small lumps of muco-pus, somewhat viscid, and later becoming numular and more easily produced. It soon becomes of a characteristic greenish grey colour with more or less froth.

Blood is very frequently present, and there may be merely rusty sputum, or a bright red haemoptysis, sometimes copious. Not infrequently the rusty sputum is the first evidence of pneumonia. When it appears after several days, it may suggest the picture of a central pneumonia.

Cyanosis and Dyspnoea.

Practically all cases of even moderate severity show marked cyanosis, doubtless due to the widespread involvement of the finer bronchial tubes. It begins early and is progressive to the end in fatal cases, while in cases which recover it may persist for many days.

Dyspnoea is usually a later symptom. At all events it is remarkable how slow the respirations are, as recorded on the charts, even when the temperature is high for days. This of course does not apply to either the fulminating types, or to those in which the signs and symptoms suggest a lobar pneumonia. The respirations may remain only slightly increased for days, which is the more surprising in view of the involvement of the finer bronchial tubes. In not a few cases the breathing is asthmatic in type, and the auscultatory phenomena confirm this observation.

Pulse.

Considering the height to which the temperature rises, it is notable that the pulse is comparatively slow in the early stages of the disease, often being no higher than 100° for many days, and the acceleration accompanies only the later period of the disease.

Mental State.

Delirium has occurred in but a small percentage of our cases, and was then only of the low muttering type. Apathy, however, is a constant feature of the picture, and patients are often drowsy from the start and difficult to rouse.

Jaundice.

This has appeared in about 10 per cent. of the cases.

Physical Signs.

The physical signs in the chest are characteristic of the disease in so far as they are varied and unreliable. Owing to the multiplicity of the pathological changes that may occur concurrently in the same lung, the accuracy with which one may determine the underlying condition is limited.

The outstanding feature of the physical signs is this, that with an extensive infiltration of the alveoli there may be no other physical signs than those of a fine bronchitis or bronchiolitis. For four or five days, or longer, from the onset of serious symptoms, one may find nothing more than a few fine rales at both bases or in the interscapular region. Signs of bronchitis dominate the picture.

Again, for a number of days there may be no other physical signs than suppressed breathing, even when the sputum is haemorrhagic ; one gains the impression then that a central pneumonia has existed, accompanied by a generalized fine or coarser bronchitis, and moderate oedema. In the fulminating cases, suppressed breathing may be the only physical evidence of involvement up to the end, though at autopsy widespread acute changes may be found.

In a number of cases where the disease was severe, and irregularly febrile for up to three weeks, there was very slight, if any, dullness, no blowing breathing, and no bronchophony, and yet there were marked dyspnoea, cyanosis, and diurnal variations of temperature between 100° and 103°. The physical signs showed evidence only of bronchitis and some oedema.

In other cases with similar irregular fever for as much as seventeen days, with dyspnoea and typical greenish sputum, the signs were those of bronchitis, with, in addition, the suppression of breath sounds in patches, but no dullness, no bronchophony, and no blowing breathing anywhere. This suppression of breath sounds may persist for many days before it is followed by even distant blowing breathing or other evidence of consolidation, while marked evidence of confluent pneumonia is always a late sign.

The suppression of breath sounds often remains for days after the temperature has returned to normal.

Percussion.

When dullness occurred it was found most frequently in the infra-scapular region, less often in the inter-scapular region or at the base. Anteriorly and at the apices dullness is quite infrequent, even late in the disease. It is rarely diffuse until shortly before a fatal termination.

Auscultation.

The most noteworthy auscultatory sign is the shortness of the inspiratory murmur, which ends abruptly and before the normal duration of the excursion. It is often more of an inspiratory grunt, while expiration is feeble and sometimes moderately prolonged. This, too, seems to be in keeping with the idea that the disease is primarily of the nature of a bronchiolitis.

As already noted, blowing breathing is nearly always a late sign. It corresponds in site to the areas of dullness, when these are evident.

The same may be said of the advent of bronchophony, which, however, is often a most valuable means of detecting areas of doubtful consolidation. Nevertheless, in many cases where autopsy revealed marked broncho-pneumonia, the vocal resonance has been unchanged.

To sum up, one may say that, save in the cases which resemble frank lobar pneumonia, the physical signs lead to the conclusion that the condition is a diffuse patchy involvement of both lungs, with considerable oedema, supervening on an invasion of the finer bronchial tubes ; later on, there is a more or less slow development of consolidation in various areas of the lung, more particularly in the upper portion of the lower lobe.

Course and Duration.

The course of the disease may be fulminating acute, sub-acute, or even almost chronic (i. e. lasting for four weeks or more), with favourable result, or otherwise. Death has occurred in four days, while in a few of our patients irregular high fever was continued for many weeks, terminating in ultimate recovery.

In the unfavourable cases the continued mild asphyxia leads to exhaustion.

Recovery usually occurs by lysis in the mild cases, by crisis in the cases simulating lobar pneumonia, and in the prolonged septic cases by all varieties of descent of temperature.

4 (*b*). PATHOLOGICAL AND BACTERIOLOGICAL FINDINGS IN FATAL CASES OF PNEUMONIA DURING THE INFLUENZA EPIDEMIC OF OCTOBER AND NOVEMBER, 1918.

BY

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The following report is based on observations made on a series of eighty-six fatal cases of pneumonia occurring at No. 3 Canadian General Hospital between October 1 and December 1, 1918. Bacteriological examination was carried out on material taken from the lungs at autopsy in sixty-seven of these.

I. Gross Morbid Anatomy.

The gross lesions seen at autopsy may be roughly divided into two groups, those occurring in intimate relation to the bronchial system, and the more diffuse processes.

The first groups include bronchitis, peribronchial abscess formation, bronchiectasis, and interstitial broncho-pneumonia.

Bronchitis.

Purulent bronchitis is evident in the great majority of cases, and in those of longer duration is usually very extensive. When the lung is first cut the bronchioles and smaller bronchi may often be seen to be filled with thick greenish yellow pus, which exudes copiously on pressure. In other cases it may be necessary to squeeze the lung to bring the pus into evidence, and a considerable search may be needed, but in most cases there can finally be found an area in which the bronchioles yield on pressure some beads or flakes of yellow white pus or muco-pus.

Peribronchial Abscess Formation.

In many of the lungs there may be seen scattered through the tissue, or more commonly in clusters in relation to areas of broncho-pneumonia, small white spots of 1 to 3 mm. diameter, which, on close examination, are seen to represent true abscess formation. Either they are solid and yield no pus on pressure, or they may yield a fine bead from the centre, leaving a solid yellow white margin,

or, again, the whole contents may be expressed, and with the aid of a hand lens, the wall of the cavity is seen to consist of bare lung tissue with no bronchial wall. These small white solid or semi-solid abscesses often have a striking resemblance, in the gross, to miliary tubercles. In a few cases there was extensive softening of the peribronchial abscesses, leading to the formation of numerous small cavities, giving a fine honeycomb appearance to the cut surface.

In three cases there were noted larger abscesses of 1 to 2 cm. in diameter, occurring in groups surrounded by intensely congested pneumonic tissue. These abscesses were either solid yellow, or had a yellow margin with a softened pink centre, and the general picture, particularly the intense vascular reaction about them, suggested the appearance of staphylococcus infarcts, but various stages between these and the smaller type described above were seen, and it was thought that they probably represented the same type of lesion, with a more virulent and rapidly spreading infection. In one case the microscopic examination showed the process to be apparently an infarction, possibly due to local inflammatory occlusion of vessels.

Broncho-pneumonia.

The type of nodular broncho-pneumonia seen in this series of cases seemed to be anatomically identical with that described by MacCallum and Cole in the pneumonias seen among American troops, following measles, and which they have called 'interstitial broncho-pneumonia'. This term very aptly describes the nature of the process, which, both from gross and microscopical examination, is evidently an extension of infection through the bronchial wall to the surrounding lung tissue,—not an infection of the alveoli by way of the natural air passages. In the earliest stage of the process there may be seen, scattered over the cut surface, small dark haemorrhagic areas of about 5 mm. diameter, which are raised above the surface, and rather firmer than the surrounding tissue. These may be isolated, or more commonly are seen in clusters. On pressure, fine beads of pus may often be squeezed from the centres of these areas. At the next stage these areas show, without pressure, a fine white spot at the centre of each, or larger spots with only a rim of congested tissue about them. The lung tissue surrounding clusters of these small areas shows congestion, oedema, and beginning consolidation. At a later and more commonly seen stage, the surrounding lung tissue is frankly consolidated, greyish with haemorrhagic mottling and forming irregular nodules of 1 to 5 cm. in diameter, containing groups of white spots representing peribronchial abscesses. These broncho-pneumonic patches may be scattered indiscriminately through the lung, sometimes so thickly as to recall the appearance of a tuberculous broncho-pneumonia, or, as often seen, may form definite wedge-shaped masses, with the base to the pleural surface, and showing extensive formation of fine abscess cavities. Again, several of these broncho-pneumonic nodules at the base of the lung may be embedded in a diffuse mass of oedematous secondary pneumonia, but the essentially focal character of the lesions is usually quite evident.

Bronchiectasis.

In some of the earliest cases the fine peri-bronchial abscesses were mistaken for dilated bronchioles, but this error was corrected on closer examination. In many cases of longer duration, however, and especially in areas of collapse, there seems to be a definite dilatation of the smaller bronchi, which are filled with copious greenish yellow pus. The lesion is usually difficult to diagnose with certainty.

In addition to the lesions having definite relation to the bronchial system, there are nearly always seen pneumonic lesions of diffuse type. Apart from localized areas of secondary pneumonia occurring about the firm patches of broncho-pneumonia, two main types of diffuse lesions were seen—a definitely lobular pneumonia, and a process showing intense diffuse congestion and oedema, often doubtfully pneumonic.

Lobular Pneumonia.

This condition has been very frequent during the present epidemic. The extent of the lesion varies greatly, but the appearance in detail is very constant. The area involved may comprise a single lobule, usually at the surface, a group of five to twenty or more lobules, or a massive area involving from a third to the whole of a lobe. The affected tissue is swollen, firmly consolidated, and, on section, pale grey, with a definitely granular surface, the texture and colour being precisely that of a croupous pneumonia in the grey stage. Even from the pleural surface, however, the lobular nature of the process can often be distinguished by the eye, and less often by the finger. In the latter case, the consolidated tissue is felt as a mass of firm individual nodules of a uniform diameter of about 1 cm.

On section, the borders of the individual lobules are usually very distinct to the eye, particularly at the margins of the consolidation, where pneumonic lobules partially or completely separated from the main mass may be seen. In some cases, each lobule stands up convexly from the cut surface, and in every case the feel of the cut surface is distinctly nodular. The pneumonic tissue is completely airless, but from the centres of the lobules there can often be squeezed fine beads of pus. In a few cases a combination of the bronchial lesions described with the diffuse lobular pneumonia was seen, clusters of peribronchial abscesses, with some haemorrhagic areas, being scattered through the affected lung tissue. Abscess and cavity formation on an extensive scale is common in these areas of lobular pneumonia, pointing to the presence of interstitial infection. A single lobule, or an extensive area involving many lobules, may be softened, grey pink in colour, and semi-fluid, leading to the formation of well-defined cavities, or to large, very irregular, serpiginous cavities embedded in partially softened tissue. It may be noted that these cases were usually recorded as having coughed up during life thick grey pink fluid of precisely the type seen in these cavities.

Sometimes, where the lobular process involves a whole lobe, and where the outlines of the lobules are less distinct than usual, it is

difficult to separate the condition from a true lobar pneumonia, but we believe that these lesions, as seen during the present epidemic, represent nearly always an infection occurring by the air passages, and truly lobular in nature. In most doubtful cases, other parts of the lungs show lesions more definitely bronchial in type.

Diffuse Congested and Oedematous Type.

Most cases showed in some part of the lung tissue a certain amount of congestion and oedema, either localized or diffuse, and probably often representing a pneumonic process, but there was a quite distinct type of case, in which death occurred after a comparatively short illness, showing intense diffuse congestion and oedema as almost the only lesion, with little or no evident bronchial involvement. When the condition is outspoken, the lung tissue may be almost airless, pieces sinking in water, but it can usually be almost completely collapsed on pressure, yielding copious, thick, bloody fluid with no evident pus. We believe, however, that the process represents a true pneumonia due to diffuse infection. In less advanced stages, and particularly where the process occurs as a secondary one in relation to focal lesions, it may appear only as congestion and oedema, with considerable air-holding tissue, pieces floating in water, and yielding on pressure only thin frothy blood-stained fluid. Again there may be seen, usually about areas of broncho-pneumonia, a diffuse pneumonia, on section grey, firm, very wet, and with a smooth shining cut surface.

In addition to the two groups of inflammatory conditions, lesions of mechanical origin are frequently seen, viz. collapse, which was fairly frequent in this series, and infarction, which was rare and indefinite.

Collapse.

The condition was seen in the cases of longer duration, and varied in extent from small patches at the free margins, to massive areas involving a considerable portion of a lobe. The marginal patches are usually about 2 to 3 cm. wide on the free edge, and extend for about the same distance into the lung. They are sharply outlined, dark in colour, and sometimes definitely depressed and leathery in consistence, but more often fairly firm, and suggesting the presence of a pneumonic process as well. We believe that they usually follow a pneumonic lesion, but that they are to be ascribed to the local plugging of the bronchi by thick tenacious pus. In nearly every case these areas on section show bronchioles distended with such exudate, and yielding large quantities of it on pressure. Scattered small areas of 1 to 2 cm. may be seen at the lung surface, which, on section, suggest a haemorrhagic process, but which microscopically show definite collapse.

More massive collapse is seen where pleural effusion is extensive, and in six cases we noted the occurrence of a type of collapse identical with that described by Colonel T. R. Elliot in chest wound cases. The area of collapse is seen externally as an irregular strip 2 to 10 cm. wide running from the upper posterior part of the lower lobe diagonally downwards and forwards, through the lower free margin and

on to the diaphragmatic surface. This area corresponds to the distribution of one of the branches arising from the main bronchus to the lower lobe, the second branch from the top. In three cases this condition was bilateral, and the remainder of both lobes showed extensive pneumonic lesions. In two cases it was unilateral, of which one was a case of chest wound, in which pneumonia with this collapse had developed on the opposite side. The tissue involved was in no case completely collapsed, but was dark red in colour, leathery, and relatively airless, with the smaller bronchi dilated and filled with thick tenacious muco-pus. The explanation of the distribution of this collapse is obscure, for in no case was there any plugging of the main bronchial branch or of its larger branches.

Infarction.

Where areas of solid lobular or broncho-pneumonia involve a free margin, this border may occasionally show a condition suggesting haemorrhagic infarction, which we ascribe to local occlusion of the vessels as part of the interstitial inflammatory process. We have noted above the occurrence of groups of abscesses, some of which suggested in the gross the appearance of septic infarcts, and one of which microscopically seemed to be of that nature.

Haemorrhagic Pneumonia.

Apart from superficial more or less well-defined areas which suggest infarcts, a number of cases showed more widely scattered and much more indefinite haemorrhagic areas, the nature of which was more doubtful. These usually were of 1 to 3 cm. diameter, with indefinite borders, and often soft and incompletely consolidated. Many of these probably represented an early stage of the interstitial broncho-pneumonia described, although no evidence of purulent bronchitis could be seen. Again, in the pneumonias of the diffuse, wet, congested type, there might be found very indefinite masses which were more solid and more haemorrhagic than the surrounding tissue. In two cases numerous well-defined, strikingly globular masses of 2 to 5 cm. diameter were seen. These on section showed uniformly circular outlines, and were composed of firm, fairly dry, dark reddish black solid tissue, bordered by pale pneumonic tissue. At the centre of each mass was a blood-vessel of 3 to 5 mm. diameter, and a small bronchus placed more eccentrically. In no case was there any thrombosis of the central vessel. Microscopic sections from the centre of one of these areas showed both vessel and bronchus free from exudate, but surrounded by a central area of interstitial pneumonia. The origin of the process could not be definitely determined.

Emphysema.

There was usually some evidence of emphysema at some part of the lungs, but it was seldom extreme, and was usually more or less masked by oedema and congestion.

Emphysematous bullae were not infrequent at the anterior free margins. One case showed bilateral local emphysema in the lower lobes, with the same distribution as the peculiar type of collapse

noted above, i.e. a strip running downwards and forwards from the apex of the lobe to the diaphragmatic surface, the lung tissue in front and behind being pneumonic.

Gross Anatomical Distribution of Lesions.

A most constant finding was the localization of the more advanced lesions in two positions :

1. At the lower margin of the upper lobe, often as a strip extending from the apex along the lower border to the anterior margin.
2. At the vertebral border of the lower lobe.

Apart from these two positions, the lesions were commonly more advanced and extensive in the posterior parts of the lungs.

The peculiar distribution of collapse and of emphysema in certain cases has already been noted.

Pleural Cavities.

Non-purulent effusion was seen in 40 per cent. of cases, and varied from 50 c.c. to a litre. In seven cases, or 8 per cent., the quantity was 500 c.c. or more. The fluid was thin, clear, and usually brownish or definitely haemoglobin stained. In 20 per cent. of cases thin, slightly purulent effusion was seen, and in three cases, or 3·5 per cent., definite empyema with thick pus.

Fibrinous or fibrino-purulent exudate occurred on the pleural surfaces in 60 per cent. of cases.

Sub-pleural haemorrhages occurred in 68 per cent. (the figure is probably higher than this, for in some cases their presence or absence was not recorded). These were usually petechial, but in a considerable number of cases there were also seen larger patches up to 3 cm. showing a delicate, thin, lace-like or flower-like haemorrhagic layer under the pleura. More dense haemorrhagic plaques were seen over areas of broncho-pneumonia. Haemorrhagic lesions were often seen on the parietal as well as on the visceral pleural surfaces.

Larynx and Trachea.

In the majority of cases the larynx was pale and neutral, but shortly below it the trachea showed diffuse congestion, becoming progressively more intense as it was followed down to the bifurcation and on into the main bronchi. Fine sub-mucosal haemorrhages were not infrequently seen. In a certain proportion, about 20 per cent. of the cases in which it was examined, the larynx also showed diffuse congestion, and these were usually cases in which laryngitis had been particularly evident during life.

II. Microscopical Examination.

The histological examination of the material obtained is as yet very incomplete, but the sections that have been studied confirm, broadly, the impressions given of the nature of the processes from gross examination. The bronchial lesions are rather more extensive than appeared in the gross, and in some cases in which it was thought that the bronchial walls were intact, the sections show at least beginning destruction of the walls. In more advanced lesions the

bronchiole and its surrounding tissue is converted into an abscess bordered only by pneumonic alveoli. The early stages of the broncho-pneumonic process show bronchioles filled with pus and more or less devoid of walls, surrounded by peribronchial connective tissue in which are numerous large dilated blood-vessels, and only occasionally a definitely haemorrhagic process. Diffuse cellular infiltration, both leucocytic and lymphocytic, is seen, and the surrounding alveoli are filled with fibrinous and purulent exudate. The later stage of the broncho-pneumonia shows a complex and inconstant picture, but the cellular exudate in the alveoli usually shows a considerable proportion of large mononuclear cells, many of which can be definitely seen to have arisen from the alveolar epithelium, which is swollen and desquamated, often as a uniform layer. In this way giant cell-like masses may be formed.

The lobular pneumonia shows microscopically less fibrin than might have been expected from the gross. Diffuse purulent exudate is seen, often with a considerable proportion of mononuclear cells, probably epithelial.

The diffuse congested wet type shows extensive oedema and haemorrhagic exudate, and usually scattered leucocytes in varying numbers, and also areas of greater leucocytic concentration.

The collapsed areas may show fairly extensive beginning overgrowth of connective tissue and blood-vessels, and giant cell-like masses may be seen.

III. Bacteriological Examination.

In making cultures from the lungs at autopsy the material which was selected by preference was the pus or muco-pus which was squeezed from the fine bronchi in the pneumonic areas. The lung was cut through, and then, while one person squeezed a favourable portion, another picked up the beads or flakes of pus with a capillary pipette as they were expressed from the bronchi. The co-operation of two workers in this way saved unnecessary contamination of the material. Where there was no purulent bronchitis evident, and in most cases where there was definite consolidation, cultures were also made from the involved areas by puncturing the external surface of the lung after searing.

The material so obtained was spread on plates made by adding about 10 per cent. of citrated blood (citrate sol. and blood—equal quantities) to trypsin agar, the plates being well dried before use. For the isolation of *B. influenzae* we much prefer to use unaltered blood. The colonies of the bacillus are undoubtedly much finer than with the various forms of altered blood (trypsinized blood, heated blood, &c.), but this character makes them more easy to distinguish from streptococci, and, in particular, colonies of pneumococci or streptococci are easily distinguished by the changes which they produce in the colour of the blood. In fact, almost the only other colonies which closely resemble those of *B. influenzae* are those of 'indifferent' streptococci, i.e. those producing no change of blood, and these were rare in the series reported. The colonies picked from the original plates were planted either on fresh blood agar, or on

trypsinized or heated blood agar, usually on a fresh blood agar plate for the first transplant as streptococci or pneumococci, the commonest source of contamination in picking the fine colonies, are ruled out without the necessity of smearing the resultant growth. Except where *B. influenzae* was greatly predominant and well isolated several colonies were usually picked. Thirty or forty such colonies may be planted on one blood agar plate by squaring off the bottom of the plate with a grease pencil.

The most striking feature of the bacteriological results in the series has been the almost constant presence of Pfeiffer's bacillus. Thus, from the lungs of sixty-seven cases examined, the organism was isolated in fifty-six, in three cases typical colonies were seen on the plates and their nature confirmed by smears, although the organism was not isolated in pure culture, and in one further case, in which the lung plate was too thickly overgrown to allow of isolation of *B. influenzae,* the organism was recovered from the heart blood. Thus the organism was certainly present in sixty cases, or 90 per cent. Allowing for errors of technique unavoidable in a series of this size, such as the occasional plate which is too thickly sown, this means that the bacillus was practically constantly present. In 45 per cent. of the cases *B. influenzae* was recorded as the predominant organism on the plates, and in many cases it was almost pure.

The diagnosis of pneumococcus was made on the presence of capsules and the fermentation of inulin. Owing to shortage of this material it was impossible to test all the possible organisms isolated, and all organisms of the pneumococcus-streptococcus group which produced a green colour on blood and which were not tested on inulin, are included in the figures given in the charts as ' Pneumococcus or Green Streptococcus '. Organisms showing no capsule and not fermenting inulin, and producing a green colour on blood, are classed as ' Green Streptococci '.

Among the figures for pneumococcus are included three cases which yielded typical *Streptococcus mucosus.* These cases were all recognized in the gross as being probably due to this organism, since we had seen two similar cases in June of this year. They showed solid massive lobular pneumonia, the cut surface of which yielded very copious thick, pinkish grey, very mucoid fluid.

A striking feature of the results has been the great rarity of haemolytic streptococci. These were isolated in only five cases, and in only three were they predominant or in sufficient numbers to justify their consideration as a prominent cause of the lesions. This is the more striking in view of the fact that several other workers have described pneumonias of apparently the same type anatomically as those seen in this series, in which the haemolytic streptococcus was almost constant, and in which the lesions noted were considered as characteristic of infection with this organism. Thus Cole and MacCallum (1), in describing the broncho-pneumonia following measles in American troops, give a picture of the areas of ' interstitial broncho-pneumonia ' which is identical with that shown by the same type of lesions in our series. They found, however, haemolytic streptococci in every case, and appear to consider this well-defined lesion as characteristic for the organism. Again, Fildes, Baker, and

Thompson (2), after describing an autopsy which they give as a type of the pneumonia in the present epidemic, say ' The organism which is immediately responsible for the above pathological picture is a haemolytic streptococcus '. The case described corresponds to those which we classed as ' diffuse, wet, congested pneumonia ', and showed also haemorrhagic masses of a type which we have noted. Among twenty-one cases of this class in our series a haemolytic streptococcus was recovered, it is true, more often than in the other types of pneumonias (see Table V), but even in these it occurred in only 14 per cent. of cases, while in thirty-seven cases showing the interstitial broncho-pneumonia of Cole and MacCallum, it was present in only 2·7 per cent.

Keegan (3) reported twenty-three fatal cases from an American Naval Hospital in Massachusetts, from 82 per cent. of whom *B. influenzae* was recovered at autopsy, while the remainder showed haemolytic streptococci. Pneumococcus, however, was the most common secondary invader (56 per cent.).

Next to *B. influenzae*, staphylococcus was the most frequently found organism. In 21 per cent. of cases it occurred in predominant numbers, usually on plates from bronchial pus.

An analysis of the relation of the type of organism isolated to the type of lesion present (Table V) does not reveal any features striking enough to definitely ascribe any type of lesion to infection with any one particular organism. The only difference worthy of consideration is the higher proportion of haemolytic streptococcus in the wet congested type. It must be remembered, however, that the total number of such streptococci isolated was very small.

Comparison of results obtained with material from various types of lesion in the same lung have also failed to give much light on this question. There was usually no marked difference between the plates made from bronchial pus and those from diffusely consolidated tissue, with the exception that staphylococci were sometimes more numerous in the bronchi. Our results have not enabled us to answer the question as to whether *B. influenzae* by itself can cause diffuse pneumonia, but in several plates from such lesions we have found it in predominant numbers, and in one case, puncture of a solid grey massive lobular pneumonia, with little bronchitis, gave a confluent growth of *B. influenzae*, pure save for six or eight colonies of pneumococcus. In this connexion it must be remembered, of course, that in the late stages of ordinary lobar pneumonia it may be difficult to isolate the pneumococcus from the affected lung.

Keegan, in twenty-three cases of pneumonia, got *B. influenzae* in pure culture six times by lung puncture at autopsy, and thinks that it was the cause of the pneumonia.

To sum up, then, we think that the constant presence of *B. influenzae* in the condition entitles it to be considered as the causative agent, even if only in the sense that it prepares the way for other invaders. We think that it is always responsible for the bronchitis, and it is probable that it may also cause pneumonic lesions by itself. The nature of the secondary infection has been too varied to admit of characterizing any one organism as a specific agent, but we think that the pneumococcus played the most important part in the

pneumonias of our series. It is not justifiable to ascribe any of the lesions noted, with the exception of the bronchitis, to one particular organism. The occurrence, with similar lesions, of different organisms in different parts of the world, would seem to indicate that the nature of the secondary infection depends largely on the relative prevalence of the various members of the pneumococcus-streptococcus group in the region in question.

REFERENCES.

(1) Cole and MacCallum, *J. Am. M. Ass.*, 1918, **70**, 1146–56.
(2) Fildes, Baker, and Thompson, *Lancet*, Lond., Nov. 23, 1918.
(3) Keegan, *J. Am. M. Ass.*, 1918, **71**, 1051.

Table I.

Relative Frequency of the Gross Anatomical Lesions in Eighty-six Autopsies.

	No.	%
Purulent bronchitis present	67	78
Purulent bronchitis pronounced	21	24
Peribronchial abscesses	38	44
Interstitial broncho-pneumonia	51	59
Lobular pneumonia present	51	58
,, ,, massive	35	43
,, ,, isolated	27	31
Diffuse wet congested pneumonia	25	29
(Of these, purulent bronchitis present in 14, or 56%)		
(Absent or doubtful in 11, or 44%)		
Collapse present	22	26
,, local	12	14
,, massive	5	6
Following distribution of 2nd tertiary bronchus to lower lobe	6	7
Haemorrhagic pneumonia (including doubtful broncho-pneumonic areas)	13	15

	No.	%
Pleural effusion, non-purulent	35	41
Pleural effusion, thin, slightly purulent	17	20
Definite empyema	3	3·5
Fibrinous and fibrino-purulent pleurisy	51	59
Sub-pleural haemorrhages	53	62

	Larynx.	*Trachea.*	*Bronchi.*
Nos. recorded	15	20	34
Congested	4(27%)	20(100%)	32(83%)

Table II.

Bacteriological Results. Direct Smears of Material from Lungs of Fifty-two Cases.

	Present.		*Predominant.*	
	No.	%	*No.*	%
Definite Gram-negative bacilli with characteristic morphology of *B. influenzae*	35	67	19	36
Organisms of streptococcus-pneumococcus group. Capsules stained	18	35	10	19
Capsules doubtful, or not examined for capsules	7	13	2	4
Capsules absent	21	40	12	23

Gram-negative cocci were seen in only a small number of cases (less than 10%). A few cases showed probable staphylococci, but it was seldom possible to be certain of these organisms.

Table III.

Cultures from Lungs in Sixty-seven Cases.

	Present.			*Predominant on Direct Plate.*	
	No.	%		*No.*	%
B. influenzae, isolated	56	84	88 %	30	45
B. influenzae, not isolated, but colonies seen on plate and smears from these typical (1)	3	4			
Staphylococcus (nearly always *aureus*)	51	72		14	21
Pneumococcus, isolated	17	25		5	7·5
Green streptococcus, isolated (2)	17	25		3	4·5
Pneumococcus or green streptococcus, undetermined (3)	23	34		5	7·5
Haemolytic streptococcus	5	7·5		3	4·5

'Indifferent streptococci', i. e. those producing no change on blood-agar, were isolated in four or five cases, never as the predominant organism.

Gram-negative cocci were seen in small numbers on a considerable number of plates, but were never predominant, or even in relatively large numbers.

Notes.

(1) In one case, in which *B. influenzae* was not found in the lung, it was recovered from the heart blood, making the total cases from which the organism was recovered at autopsy 60 or 90 %.

(2) Under 'green streptococcus' are classed the organisms which produce a green colour on blood-agar, have no capsules, and do not ferment inulin.

(3) Owing to our supply of inulin having become exhausted, a number of organisms of this group could not be tested.

Table IV.

Cultures from Heart Blood in Fifty Cases.

Positive, 25 or 50 %	*No.*	%
B. influenzae, isolated	2	4
Pneumococcus	4	8
Green streptococcus	1	2
Pneumococcus or green streptococcus, undetermined	6	12
Haemolytic streptococcus	2	4
Staphylococcus aureus	8	16

In the remaining two positive cultures organisms suggesting *B. influenzae* were seen, but were not isolated.

Table V.

Relation of Organisms isolated to Type of Lesion present.

	Purulent Bronchitis.	*Peri-bronchial Abscesses.*	*Interstitial Broncho-pneumonia.*	*Lobular Pneu-monia.*	*Wet Congested Type.*	*Total Cases.*
Numbers examined.	52	30	37	39	21	67
B. influenzae	92 %	97 %	95 %	85 %	76 %	88 %
Staphylococcus	81 %	77 %	84 %	77 %	76 %	72 %
Pneumococcus	30 %	20 %	22 %	31 %	27 %	25 %
Green streptococcus	25 %	33 %	32 %	23 %	19 %	25 %
Pneumococcus or green streptococcus, undetermined	33 %	37 %	32 %	31 %	29 %	34 %
Haemolytic streptococcus	6 %	6·6 %	2·7 %	10 %	14 %	7·5 %

5. REPORT ON THE BACTERIOLOGY AND PATHOLOGY OF 46 FATAL CASES OF INFLUENZA

BY

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WITH A NOTE ON THE PREPARATION OF MEDIA FOR THE CULTIVATION AND STUDY OF *B. INFLUENZAE* (PFEIFFER)

BY

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AND
Miss S. E. Williams, A.A.N.S.

During the past month 63 fatal cases of 'influenzal' bronchopneumonia occurred in No. 5 General Hospital.

When our bacteriological methods of investigation became stabilized, a series of 46 consecutive cases was submitted to analysis. The results are summarized in the succeeding tables.

I. Methods.

Smears were made from the bronchi and from portions of lung which exhibited haemorrhagic engorgement, consolidation, pus, or any other abnormal feature. The smears were stained by Gram's method, and the findings noted. Cultures were made on rabbit's blood-agar plates, subsequent subcultures being made on slopes of the same medium. A series of preliminary experiments showed that rabbit's blood was superior to human blood for growing Pfeiffer's bacillus. In studying the organisms present in lung or bronchi, a series of strokes was made on the blood-agar plates, and after incubation for 18 to 24 hours the colonies were examined and subcultures made. Blood cultures from the heart were also made in 44 cases.

In the earlier cases an attempt was made to study the organisms occurring in parts of lung tissue exhibiting different stages of bronchopneumonia. The results indicated that this was not a fruitful line of investigation, and was discontinued.

II. Summary of Results of Bacteriological Examination.

1. *Blood Cultures.*

No. of Examinations made.	*Organism recovered.*				
	B. Influenzae.	*Pneumococcus.*	*Streptococcus.*	*Staphylococcus.*	*No Growth.*
44	1 case	12 cases	1 case	1 case	29 cases

2. *Bronchi and Lungs.* Table showing the organisms seen in direct smears from the organs, or subsequently isolated on cultivation.

No. of Examinations made.	*Organisms recovered.*			
	B. Influenzae alone.	*B. Influenzae + Pneumococci.*	*B. Influenzae + Streptococci.*	*B. Influenzae + Pneumococci. + Streptococci.*
46	8 cases	19 cases	3 cases	15 cases

With one exception, *B. influenzae* was recovered from every case, either alone or associated with the pneumococcus, or streptococcus, or both.

In addition a Gram-negative coccus was seen or isolated in 29 cases; it was always associated with one or more of the organisms mentioned above.

3. Table showing the relative frequency with which the various organisms were seen in direct smears, or cultivated from (*a*) the lungs, (*b*) the bronchi.

	B. Influenzae.	*Pneumococci.*	*Streptococci.*	*Gram-negative cocci.*
Lungs	36	26	14	14
Bronchi	42	28	17	24

4. Summary showing the bacteriological results of the microscopical examination of direct smears, and of cultivation on blood-agar, of the lung and bronchi of 46 cases.

No. of Cases.	*Lung.*								*Bronchus.*							
	Direct Smear.				*Culture.*				*Direct Smear.*				*Culture.*			
	B. Influenzae.	*Pneumococcus.*	*Streptococcus.*	*Gram-negative cocci.*	*B. Influenzae.*	*Pneumococcus.*	*Streptococcus.*	*Gram-negative cocci.*	*B. Influenzae.*	*Pneumococcus.*	*Streptococcus.*	*Gram-negative cocci.*	*B. Influenzae.*	*Pneumococcus.*	*Streptococcus.*	*Gram-negative cocci.*
46	14	14	2	10	36	23	14	7	25	21	3	25	40	14	17	13

5. The following chart shows the relationship of the bacteriological findings in the bronchi and lungs with reference to the duration of illness.

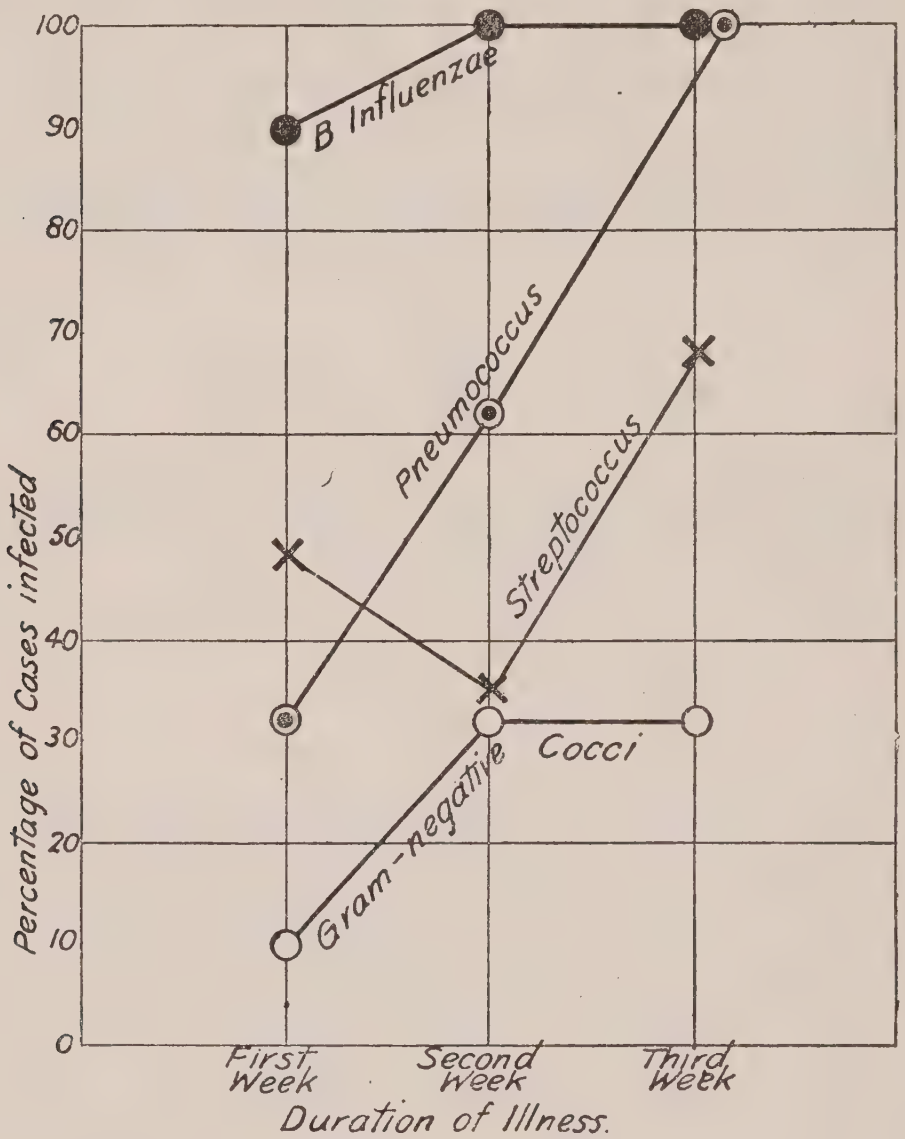
It will be seen that, while *B. influenzae* is practically constant, infection with pneumo- and streptococci increases in cases of longer duration; the Gram-negative cocci are apparently of small importance.

III. General Post-mortem Appearances.

Often frothy sanious fluid was exuding from the mouth and nostrils. The veins of the neck were engorged and full of dark fluid blood. On opening the thoracic cavity, the front of the turgid

lungs pushed up, usually full of air and crackling ; and often rupture of air vesicles had taken place, giving patches of acute emphysema, pallid beneath the pleura. There were frequently small areas of subpleural haemorrhages.

In 4 cases there was a considerable amount of dark straw-coloured fluid free in one pleura. In these cases, the pleura affected had patches of soft greenish-yellow thick fibrin scattered over the lower parts of the lung and between the lobes. Twenty-four other



cases showed recent soft fibrinous adhesions scattered over one or both lungs ; in some instances these were very dense, although recent, and in tearing through them quantities of blood-stained fluid exuded from the mouth and nostrils, as it was expressed from the lung and bronchioles. Three other cases had old firm fibrous adhesions of a previous pleurisy.

Total recent pleural involvement, 28 cases (60 per cent.). In the remaining cases the pleurae in front were pale, showing sometimes emphysema and petechial haemorrhages as described above ; and posteriorly, over the engorged or consolidated lung, the pleural surface had lost its glistening appearance, and was of a dark plum colour.

Lungs.

The most striking feature was the general engorgement and waterlogged condition of the lungs ; except in the grey consolidated patches, there was a profuse exudation of frothy sanious fluid from the cut surface. In extricating the lungs, especially when pleural adhesions were present, frothy blood-stained fluid was expressed from the bronchi and poured from the mouth.

1. To this primary *inflammatory oedema and congestion* were added the following types of broncho-pneumonic involvement.

2. *Peribronchial type.* In the early stages this condition showed as small bright-red spots of consolidation about 5 mm. in diameter surrounding a small bronchus. On palpating the lung through the pleura or passing the finger over the cut surface, the impression was given of small knots in the lung of firm consistency, resembling the feel of miliary tubercles. This condition frequently remained limited in extent, becoming grey and later softening, so that in the late stages the lung surface was pitted with small discrete abscesses.

3. *Broncho-pneumonic type.* Here the cut surface of the lung amidst general engorgement and oedema showed firmer raised bright-red areas varying in diameter up to 25–40 cm. Later these areas became larger, dark-red, and confluent. In the next stage greyish-red patches were evident, and in some instances the confluent greyish-red massive areas resembled lobar pneumonia, but the lobe showed places in various stages from dark-red areas to parts where softening and abscess formation was taking place. In some instances the whole alveolar part of the lung was diffluent, and abscesses up to 50 cm. in diameter were present, full of broken-down lung tissue and traversed by strings of more resistant bronchi.

4. *Purulent bronchitis.* From parts of the lung in all stages of involvement worms of yellowish pus could often be expressed from the small bronchi.

5. *Acute emphysema.* Along the free borders of the lungs the air vesicles were frequently ruptured and confluent.

In one case, whose bronchitic signs dated from only two days before death, only one small area of bright-red consolidation near the hilus of the left lower lobe was found in addition to a tremendous haemorrhagic engorgement of the whole of the lungs. In all other cases protean combinations of the pathological varieties cited above were found throughout the lungs. On the whole, however, the parts dependent in the dorsal decubitus showed the most widespread and farthest developed involvement.

In one case only a small infarct was discovered ; it was situated on the anterior border of the left upper lobe. Two cases showed definite evidence of tubercle, one an old calcified nodule in the apex of the right upper lobe, the other an acute miliary tubercle scattered widely throughout the lungs. In the latter, tubercle bacilli occurred in the smears, and cultures showed the presence of *Bacillus influenzae.*

Respiratory Tract.

In all cases the large bronchi contained frothy bloodstained fluid, and showed congestion of the mucous membrane. In many cases

this congestion was intense, and extended up to and involved the epiglottis, being accompanied at times by submucous haemorrhages. In cases of longer standing, erosion and ulceration of the vocal cords had occurred.

Heart.

The cavities of the right side were always much dilated and flabby, distended with dark blood, or often with firm white clot extending to the root of the pulmonary artery. The left ventricle was usually firm and contracted; but in 13 cases (30 per cent.) the muscle of the left ventricle was also softened and flabby. No acute involvement of the valves was observed. In only one case were sub-pericardial haemorrhages noted; and no instance of excess of fluid in the pericardium or of pericarditis occurred.

Liver.

The liver was always considerably engorged. A constant observation was the presence of patches of degeneration in the liver. In cases examined even within two or three hours of death, small subcapsular areas of yellowish degeneration occurred, principally on the upper surface of both lobes and at the free anterior border, extending sometimes to a depth of 2–3 cm. In some instances the degeneration was widespread throughout the liver, but this may have been due to early post-mortem changes. In two cases recent fibrinous adhesions of the diaphragm to the upper surface of the right lobe had occurred.

Spleen.

The spleen was small and firm in 22 cases, softened in 14 cases, and large and softened in 10 cases.

Adrenals.

The adrenals in 20 cases were observed to be friable; in one case both adrenals showed haemorrhagic involvement of the whole gland.

Kidneys.

The kidneys, beyond general engorgement, and some oedema of the cortex, showed acute involvement in only one case, in which there was a recent exacerbation of a chronic nephritis.

Stomach and Intestines.

In one case with a clinical history of haematemesis, the gastric veins were distended and there were numerous submucous haemorrhages on the stomach wall. In the remainder, these organs appeared normal.

Brain.

In one early case with symptoms of meningism, the brain and medulla presented no macroscopic abnormality, and the cerebrospinal fluid on culture remained sterile.

The post-mortem examinations were made at No. 5 General Hospital by one of us (S. W. P.); and the bacteriological work was carried out in the laboratory of No. 25 Stationary Hospital by the other two (E. M. L. and F. E. W.).

NOTE ON THE PREPARATION OF MEDIA FOR THE CULTIVATION AND STUDY OF *B. INFLUENZAE.*

The experiments described below were carried out towards the end of 1918, when the epidemic of influenza, which had been prevalent, was subsiding.

In the previous epidemic, in June 1918, during which some fifty cases of influenza were investigated in this laboratory by Lieutenant-Colonel C. J. Martin, F.R.S., A.A.M.C., the medium used for the isolation of *B. influenzae* consisted of agar to which unheated citrated rabbit's blood had been added. A suitable piece of tracheal mucus was selected, washed in saline or water, and sown on rabbit's blood-agar plates and incubated for eighteen to twenty-four hours at 37° C. On this medium *B. influenzae* appeared as very small, hemispherical, perfectly clear colonies. After a little practice in the preparation and use of this medium, little difficulty was experienced in growing *B. influenzae* from sputum from patients suffering from the disease.

When the second wave of the epidemic began in this Base in the autumn of 1918, a similar technique was adopted in order to cultivate, if possible, *B. influenzae* from sputum, and from autopsy material from influenza cases. In the earlier stages of our observations at this time we found that we were not so successful in isolating the organism as in June. Frequently we failed to obtain a growth of the bacillus from material which, as microscopical examination of stained smears showed, contained minute gram-negative bacilli, morphologically resembling the influenza bacillus of Pfeiffer, in great numbers ; and, moreover, when colonies of *B. influenzae* developed on our plates they were usually few in number. The one point of difference which had been introduced into the technique was that whereas in June rabbit's blood was used exclusively, in these earlier experiments, which met with only a limited success, we used human blood. Our earliest experiments were made with blood, and blood-agar, which had been supplied to us from the pathological laboratory of a venereal diseases hospital, the blood having been obtained from patients. This medium, although excellent for the cultivation of other organisms, was found to be of little value for the cultivation of *B. influenzae.* We thought, at the time, that possibly the fact that some of the patients had received intravenous injections of salvarsan might account for the small success we met with when using the medium, but we found later that no better results were obtained when blood from healthy, normal human beings was used. We then returned to the original method of June, as used by Martin, and used citrated rabbit's blood and encountered no further difficulty in isolating *B. influenzae* from our material.

It appears, therefore, that in isolating *B. influenzae* the species of animal from which the blood required for the media is derived is a factor of some importance, and may possibly account for the failure which some workers have experienced to isolate *B. influenzae* from material which microscopical examination shows to be heavily infected with the organism. A series of experiments was therefore carried out with a view to studying this, and two other, points.

It was suggested to us that the addition of potato extract had been of value in the isolation and cultivation of the gonococcus and certain other organisms, and that the addition of potato extract to our blood-agar might provide a medium suitable for our purpose. This was tried.

It has been known for some years that media containing *heated* blood yields more profuse growths of *B. influenzae* than similar media containing *unheated* blood. The earlier observations on this point have been repeatedly confirmed, and quite recently media containing 'changed' blood have been prepared and studied in greater detail by Fleming, Levinthal, and Matthews.

The following batches of media were made, and their value for the isolation and cultivation of *B. influenzae* studied and compared.

(i) Agar and unheated citrated human blood
(ii) ,, ,, ,, ,, rabbit's ,,
(iii) ,, ,, ,, ,, horse ,,
(iv) ,, ,, ,, ,, human blood and potato extract.
(v) ,, ,, ,, ,, rabbit's ,, ,, ,, ,,
(vi) ,, ,, ,, ,, horse ,, ,, ,, ,,
(vii) ,, ,, heated ,, human blood
(viii) ,, ,, ,, ,, rabbit's ,,
(ix) ,, ,, ,, ,, horse ,,
(x) ,, ,, ,, ,, human blood and potato extract
(xi) ,, ,, ,, ,, rabbit's ,, ,, ,, ,,
(xii) ,, ,, ,, ,, horse ,, ,, ,, ,,

The media containing the heated blood (Nos. vii–xii) was prepared in the following way. Tubes containing 5 c.c. of nutrient agar were melted and allowed to cool to 60° C. and 0·2 c.c. of citrated blood added to each tube, the tube shaken gently to secure a homogeneous mixture, and then transferred to another bath at 80° C., and left there for a few minutes until the contents of the tube became chocolate in colour. The tubes were then sloped. In preparing the media containing the so-called 'unheated' blood the melted agar was cooled to 50° C. and the citrated blood added and the tubes sloped at once. This latter medium is of a bright red colour.

On the twelve varieties of media thus prepared the growth of ten strains of *B. influenzae* was studied. The results of our observations are summarized below.

(i) *B. influenzae* grows much more profusely on media containing blood which has been heated to 80° C., and the colonies which develop on such heated blood-agar are invariably larger than those which grow on unheated blood-agar.

(ii) A better growth is obtained on media containing unheated rabbit's blood than on media containing unheated horse blood, and this latter yields better growth than media containing unheated human blood. In six of the ten strains studied no growth occurred on the unheated human blood-agar media, and the growth on the remaining four cases was extremely scanty. Three out of the ten strains failed to grow on the unheated horse blood-agar, and growth was obtained in nine out of the ten strains sown on to unheated rabbit's blood-agar. In the corresponding media containing heated blood, a good growth was obtained in nearly every case.

(iii) The addition of potato extract did not affect in any way the cultural value of the various blood-media tested.

(iv) On numerous occasions we observed that strains of *B. influenzae* cultivated on media containing unheated blood, on examination showed bacilli of the filamentous variety. Subcultures of these involution forms on to heated blood-agar media resulted in a growth of organisms morphologically true to type.

In this laboratory we make use of media containing either unheated or heated blood, according as to whether (i) sputum or autopsy material is being examined for the presence of *B. influenzae*, or whether (ii) a previously isolated and identified strain of *B. influenzae* is being dealt with. It is now our practice to sow sputum or other material on plates of unheated rabbit's blood-agar. The blood being added to the melted agar at a relatively low temperature (50° C.), such plates have a bright red appearance, and the unchanged haemoglobin which they contain serves as a valuable 'indicator', and is an aid to the picking off of colonies of *B. influenzae*, and to the differentiation of them from colonies of pneumococci and haemolytic streptococci. If, on the other hand, a large quantity of influenza organisms is required (e.g. for the preparation of vaccines, for the study of the agglutination reaction, &c.), we use media containing 'changed' blood, as on these media colonies are much larger and growth is more profuse. When using heated blood there is not much to choose between the rabbit and the horse, but with human blood the results are not quite so good; in our experience Levinthal's medium or Matthew's medium are even better.

We have also carried out experiments in order to discover, if possible, a medium which will preserve the bacillus of influenza in a viable condition for some time. We find that the following medium keeps the bacillus alive for a period of sixteen days, and twelve out of fifteen strains were found to be still alive when examined twenty-one days after inoculation. The basis of this medium is nutrient agar containing 2 per cent. of starch: this is tubed in 5 c.c. quantities, melted, and cooled to 60° C. To each tube 0·2 c.c. of citrated blood (the species of animal from which the blood is drawn is immaterial) is added, the whole gently shaken and immersed for a few minutes in a bath at 80° C., until the contents become chocolate in colour. The tubes are then put into a rack and allowed to cool in an upright position. The tubes are inoculated with two or three stabs, incubated for twenty-four hours at 37° C., and the plugs then paraffined and the tubes returned to the incubator, where they are kept.

6. REPORT ON THE MORBID ANATOMY OF INFLUENZA

BY

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The present remarks concern the autopsies on a series of 143 cases dying from influenza or its complications during October and November, 1918. It is much regretted that more were not examined, but during the height of the epidemic it was not possible to examine more than one in three or four.

It was originally intended that Captain W. Rolland, R.A.M.C., should investigate the bacteriology of the post-mortem material, and in a great many cases he did, but his early recall to civil life has put an end to his work. It may be possible to publish his work later, together with some detailed histology of the lesions described below.

I. General Considerations.

The striking features revealed by these post-mortems are three:

The constancy of pulmonary lesions.
The constancy of myocardial changes.
The frequent occurrence of haemorrhages.

Very varying amounts of pneumonia were found, and often the amount seemed quite inadequate as a cause of death, but the myocardial condition was hardly ever such as easily to be overlooked, and amply compensated for the small amount of pneumonia. The presence of haemorrhage in various situations, and notably in the lungs, is probably evidence of a widespread and severe toxaemia.

Comparatively few of these fatal cases could be regarded as below the average in physique, and most were robust and apparently previously healthy men.

Apart from the disease itself the chief debilitating factor was tuberculosis, and that did appear to be present in a higher proportion of cases than previously found. In this series it occurred in twenty-one cases or 14·7 per cent. (the proportion was 9·6 per cent. in a previous series of 1,500 cases, chiefly dying from wounds). In 10 cases the lesion was pulmonary, but active in only 2. Glandular tuberculosis was present in 11, the abdominal and thoracic glands being involved in about equal numbers.

One patient had been poisoned by phosgene and another by 'mustard' gas, and two were suffering from slight wounds of the limbs. Acute appendicitis was found in one, and chronic bronchiectasis in another. Nephritis older than the present disease was found in five cases.

The morbid anatomy of these cases is best described under the following regional headings: lesions found in the pleura, lungs, mediastinal glands, heart, liver, spleen, kidneys, and brain, and a few other lesions not admitting of a grouped classification.

II. Lesions Found in the Pleura.

Pleurisy was found as follows:

	Old.	*Old, assoc. with T.B.*	*Recent.*	*Serous Effusion.*	*Empyema.*
Bilateral . .	5	4	57	8	4
Left . . .	11	4	18	6	9
Right . . .	21	7	20	3	3
Total . .	37	15	95	17	16
		Included in previous column.		Included in the previous column.	

Total effusions (serous and purulent) = 33, or about one in three of all recent pleurisy.

The old pleurisy was met with chiefly in the form of adhesions over the lower lobe—a few cases showed apical adhesions only, or were universal.

Recent pleurisy was found—usually dry—over the lower lobes and behind, often extending over the upper lobe, but seldom much in front of the mid-axillary line. A certain amount of lymph exudate was commonly seen on the surface of the lung. The parietal pleura almost always showed vascular dilatation and roughening of the surface when visceral pleurisy was present.

Effusions were generally small—viz. half to one pint—in the case of serous effusions, and up to over two pints in one case of empyema. Clear effusions were, no doubt, in some cases referable to cardiac failure, for it was not uncommon to find them associated with absence of dullness of the lung surface.

Haemorrhagic effusions were seldom seen, and never in marked degree.

III. Lesions of the Lung.

Conditions ranging from profuse oedema to lobar consolidation have been found in the lung. Whatever else was found, oedema was one of the prominent features, and the oedema was of a peculiarly slimy character in many cases in which the consolidation was extensive.

The types of pneumonia found may be classified as follows and considered separately:

A. Haemorrhages and haemorrhagic pneumonia.
B. Broncho-pneumonia—or capillary bronchitis.
C. Miliary pneumonia or bronchiolitis.
D. Lobar pneumonia.

A. *Haemorrhage and Haemorrhagic Pneumonia.*

In most cases broncho-pneumonia was accompanied by haemorrhage, present either as small round subpleural petechiae scattered over

the surface of a lobe and roughly proportional in amount to the degree of consolidation, or as larger masses of interstitial haemorrhage, which closely resembled red infarcts when near the surface of the lung. The latter type has been observed to follow the line of a vessel as if produced by leakage from various points along its course.

Broncho-pneumonic patches have often been found surrounded by a zone of the more usual consolidation—suggesting that the haemorrhage preceded the consolidation and provided pabulum for the rapid growth of organisms. (Organisms of a haemoglobinophilic type, such as Pfeiffer's bacillus, readily grow in such surroundings.)

Further, cases of death after short illness were more prone to show obvious haemorrhage than those of longer standing. A very characteristic type of lesion was one in which the lung was bulky from emphysema, and showed numerous plum-coloured haemorrhagic areas, varying in size from the size of a shilling up to a third of the lower lobe—the latter being produced by coalescence of smaller areas.

Discrete lesions resembled infarcts on section, and seen through the pleura were often of polygonal shape. They were quite solid, and on microscopic section were clearly due to haemorrhage. There was no obvious cause for infarction in these cases, and, though no microscopic evidence is adduced, were probably due to leakage from vessels the walls of which were unduly permeable.

This condition was present in early cases, and in cases showing the more profound toxaemia. In a few cases watched clinically haemoptyses were found from time to time. These were the cases which most constantly showed the presence of influenza bacilli—both in the sputum and in cultures made from the lung post-mortem.

B. *Broncho-pneumonia.*

Broncho-pneumonic areas of greater or smaller extent were the most common pulmonary post-mortem finding. They tended to coalesce, and generally there was compensatory emphysema, most often seen in the anterior parts of the lobes, but a finer mixture of emphysema, consolidation, and collapse was often seen, as in the broncho-pneumonia or capillary bronchitis of children. Sometimes it was hard to distinguish in these cases between confluent broncho-pneumonia and lobar pneumonia, but probably the latter was not common. In a fair proportion of cases of broncho-pneumonia necrosis of the lung was present, generally of small areas, but occasionally these were found to have blended into extensive branching abscesses. Almost all cases showed pus in the bronchi, and on section small beads of pus oozed from the finer tubes. This was not found in consolidated areas only, but occurred in what otherwise seemed to be fairly normal lung, recalling the purulent bronchitis of two years ago. (*Lancet*, 1917, ii, 41.)

The trachea was quite often much injected, especially in its lower part, and occasionally a slight membrane or pseudomembrane was found partially detached, but much more often there was a slimy mucopurulent secretion which welled up from the main bronchi. The injection was seldom extremely marked as in poisoning by gas or other respiratory irritants, and seldom extended much above

the larynx, although in some cases the fauces and pharynx were markedly involved. Injection was most prominent in haemorrhagic cases.

Altogether broncho-pneumonia was present in 113 cases. In 77 excessive oedema was noted; 48 showed marked purulent bronchitis, and in 51 compensatory emphysema was prominent.

Necrosis or abscess of the lung occurred 12 times; in 4 it was the obvious origin of empyemata, but in 2 there was no pleurisy, as the areas of consolidation involved were deeply situated.

The figures just given include also those under the heading of haemorrhagic pneumonia, as the possibility of separate consideration of these cases did not become apparent until too late to place them in a category by themselves. Interstitial haemorrhages and subpleural petechiae were noted as follows:

Interstitial haemorrhage	68
Subpleural petechiae	54
Both	24

Of 12 cases of necrosis of the lung haemorrhage was found in 10.

C. *Miliary Pneumonia.*

This type of pneumonia was fairly often found, and is associated with plugging of bronchioles with pus and fibrinous exudate. A small area of consolidation, with leucocytes, epithelial cells, and fibrin in the exudate, forms around the affected tube, and gives rise to a very characteristic macroscopic condition, which, however, is much more easily felt than seen. When the lung is handled it reminds one of a bag of peas, each nodule feeling rather smaller than a pea, and the surrounding lung quite soft and non-resistant. This condition is generally called bronchiolitis, but as definite consolidation of the lung is present, the name miliary pneumonia seems more appropriate.

Miliary pneumonia was found in twenty cases—14 per cent. of all cases or 18 per cent. of all broncho-pneumonia. It occurred as described in discrete form in twelve cases, and in a condition of becoming confluent into larger areas, or associated with ordinary broncho-pneumonia, in the remaining eight. Thirteen cases showed haemorrhage, eleven showed a marked degree of emphysema, and pleurisy was present in thirteen. Bilateral in eleven. Two small empyemata were found in cases of miliary pneumonia.

It was thought that this type of pneumonia might be more definitely associated with the presence of Pfeiffer's bacillus than the more normal broncho-pneumonia, because of its obvious relationship to purulent bronchitis, but this bacillus was not constantly found. Pneumococci and streptococci were found as frequently.

D. *Lobar Pneumonia.*

The lobar distribution of consolidation was not common. It was found in fifteen cases, which may be subdivided as follows:

(*a*) Almost certainly confluent broncho-pneumonia .	7
(*b*) Showing broncho-pneumonia of the other lung .	4
(*c*) Probably true lobar pneumonia	4

Only two showed empyemata—both of type *b*. Three showed serous effusions (all left-sided)—two belonged to type *b*, and one to type *c*, the latter being a very small effusion, and turbid, though not obviously purulent.

Consolidation was always present in the grey stage though a few cases showed some areas of consolidation of more recent date, especially in the upper lobes.

The cut surface of these lungs constantly showed a very wet and slimy condition, and was not of such a granular or 'ground glass' appearance as is found in typical lobar pneumonia.

A large amount of oedema was also present in seventy-seven cases of broncho-pneumonia, and in five it was the only pulmonary lesion found.

IV. Lesions of Mediastinal Glands.

Enlargement of glands at the roots of the lungs and around the lower end of the trachea was very often found, and more often during the latter part of the investigation than at first—probably because they were more frequently examined. Altogether they were found to be affected in ninety-one cases, of which twenty occurred in the first forty-three and seventy-one in the final hundred. In all probability 75 per cent. is near to the actual proportion in which they were involved.

These glands, and in a few cases the abdominal, inguinal, and cervical glands, showed much swelling due to congestion. In some cases this was absent, and the glands were pale, soft, and even breaking down, especially when there was a necrotic patch in the lung.

It is possible that the enlargement of these glands accounts for the clinical observation of a cough closely resembling whooping cough—namely a series of expirations followed by a single inspiration, in repeated spasms and in a few cases ending in a small vomit. This has been observed many times, and the state of the mediastinal glands found post-mortem suggests a causation similar to that of the cough met with in children affected by tuberculosis of these glands.

V. Lesions of the Heart.

It was very unusual to find a heart in these cases of influenza death that could be considered normal. Dilatation of the right side was the rule, and of the left extremely common. The myocardium was generally paler than usual, often showed early fatty change, and was almost always soft. Sections in the fresh state stained with Soudan III showed intense fatty degeneration. The condition of the heart muscle fully bears out the clinical observation of low blood pressure. It would be curious if such hearts could sustain a normal intraventricular pressure. The blood pressures of a ward of thirty-five patients, all in the first week of the disease, gave an average of 93 mm. Hg, the lowest being 84 mm. (taken by a dial instrument, and checked by normal controls). The pressure was found lowest at about the third or fourth day and in severe cases took several

weeks to recover. Unfortunately the pressure just before death was not taken in any of these cases. In two cases of post-influenzal depression or psychasthenia the pressure was 10 mm. below normal as much as eight weeks after the attack of influenza.

Dilatation was found in 138 cases. Myocardial pallor or softening or both in 135 cases.

In many cases the endocardial half of the muscle was very noticeably paler than the outer half. This was much more marked in some cases than in others, and was found especially in early fatal cases, and in those showing the greatest dilatation. The reason is presumably to be found in the vascular supply to the heart muscle—the smaller branches of the coronary arteries being furthest from the vessels' origin on the outside of the heart, and the part supplied by them on the inner side most affected.

The heart, like the lungs, showed subserous haemorrhages, but by no means so commonly. They were noticed in sixteen cases, in the form of small discrete rounded subpericardial haemorrhages. Occasionally small endocardial haemorrhages of a similar type were found, but with a tendency to be more irregular, or flame shaped, resembling those found in the retina in other conditions.

Pericarditis and endocarditis were not found at all. The average heart weight was 12 oz. The largest 16 oz. and the smallest 8 oz.

VI. Lesions of the Liver.

In most cases there was found some fatty change in the liver, often taking the form of irregular areas, visible beneath the capsule, and on section, of yellowish colour, and surrounded by normal looking liver. In some cases the whole liver was pale and very obviously fatty-enlarged, and with a rounded instead of a sharp anterior edge. In some cases the left lobe seemed more affected than the right, but usually they were affected equally.

In a considerable number congestion was present—doubtless accompanying a gradually failing heart, and when present together a 'nutmeg' appearance was produced.

Nothing abnormal was noticed in the gall bladder or bile ducts. Fatty change in the liver was found in 130 cases, congestion in forty-nine cases, and both were present in thirty-eight cases.

The average liver weight was 68 oz.—the largest being 109 oz. and the smallest 41 oz.

Jaundice of a non-obstructive type was found in five cases. These all showed evidences of severe toxaemia. Jaundice was not deep in any of them.

VII. Lesions of the Spleen.

The condition of the spleen is always very difficult to describe, but two main types can be mentioned in this series of cases apart from congestion, which may be present as well.

(1) The malpighian bodies may stand out prominently on cut section, as in the 'sago' spleen, the rest of the organ being either pale or congested, which latter makes the pallor of these bodies

more apparent. The spleen is usually enlarged, flabby, and often very soft. When not congested the capsule is often wrinkled.

(2) The spleen as a whole may be pale and flabby—not soft—the consistence reminding one rather more of gelatin or india rubber. It is not nearly so friable as the preceding type, and on section presents a much more uniform appearance. The malpighian bodies do not appear enlarged, and need looking for. The organ is just as often enlarged as the preceding. Enlarged malpighian bodies were found in forty-five cases, the pale flabby spleen in sixty-eight cases, and congestion was present in fifty-eight cases.

The average spleen weight was 6·6 oz. The largest spleen weighed 19 oz. and the smallest 3½ oz.

VIII. Lesions of the Kidney.

Macroscopically the kidneys almost always showed some changes which were probably attributable to the action of toxins passing through them. On the average they were a little larger than usual, and were certainly paler and softer.

Generally the cortex was a little swollen, and there were streaks, indicating areas of congestion, upon a pale or yellowish background. The bloody kidney of acute nephritis was never seen. The medulla was of a darker colour, and often showed marked congestion. The capsule stripped off easily, with a few exceptions where there was nephritis of longer standing, and the surface exposed showed distended stellate veins upon a pale smooth background. The renal substance was often quite friable. These are the appearances of what might be termed the ' toxic ' kidney, and were present in 130 cases.

Microscopical sections showed lesions which fall in line with the gross morbid anatomy.

Glomeruli were not involved except for some terminal congestion. The brunt of the damage was borne by the tubules—and especially the convoluted tubes and ascending limb of the loop of Henle. These showed desquamation with epithelial casts in situ, and in several cases a great deal of fatty degeneration was present. Hyaline was constant in the few cases it was possible to examine at the time.

About fifty cases showed, either in addition to the toxic state or apart from it, a considerable amount of congestion, which is not surprising as a result of failure of the right side of the heart.

Subacute or chronic nephritis—probably of much older date than the terminal influenza—was found in five cases only, and in all it was of the glomerulo-tubal type. The granular kidney was not seen.

Haemorrhage under the mucous membrane of the renal pelvis was seen three times, in the form of small closely crowded points as large as a pin head.

The average kidney weight was greater than normal—12·3 oz. the pair.

Oedema of the extremities, hydrothorax, ascites, or lumbar oedema were not seen except in one case of subacute nephritis, in which a small hydrothorax was found.

IX. Lesions of the Brain.

The brain was only examined in twenty-two cases and in those because some special cerebral symptoms were present.

The conditions found were as follows :

Meningeal oedema	11
Great congestion	3
Encephalitis	2
Meningitis	2
Hydrocephalus	1
Natural	3

The degree of oedema was often high and well in agreement with the condition of meningism from which the patients suffered.

Encephalitis was present in two cases and showed itself as multiple punctate haemorrhages studded throughout the white matter of the cerebrum, basal ganglia, pons, medulla, and cerebellum. The grey matter appeared not to be affected. The individual haemorrhages were small, but varied somewhat in size, and in some places were noticeably more abundant than in others ; collections were especially seen near the posterior end of the corpus callosum. As only two cases were examined no generalizations are possible, but the constant affection of the white matter is remarkable. The condition most nearly resembling what was seen is the state of the brain in poisoning by phosgene gas.

The two cases of meningitis showed no influenza bacilli on culture. One was caused by the meningococcus, and the other by the pneumococcus. The condition was in no way remarkable.

In one case in which headache had been a very prominent symptom inflammation of the frontal sinuses was found and the influenza bacillus found in the pus they contained.

A moderate degree of acute internal hydrocephalus was found in one case. No cause for this was found, the base of the brain appearing clear—meningitis was absent. During the last few hours of life this patient had a persistent spontaneous clonus of the right leg below the knee, but no localizing cerebral lesion was found.

X. Other Post-mortem Findings.

Haemorrhages have been found in other situations than those mentioned—the lungs, heart, and brain.

The lowest segment of the rectus abdominis muscle was haemorrhagic in eleven cases. At first excessive coughing was thought to be a possible cause of this, but a section cut by Captain Bashford, R.A.M.C., showed that an extensive myositis was present, with degeneration of the myelin, and proliferation of the sarcolemma cells.

Mesenteric haemorrhages were noticed once.

Intestinal haemorrhage occurred twice, and haemorrhage in the stomach once.

A gross haemorrhage into the tissue of the spleen was seen once.

Parotitis was seen in one case—associated with encephalitis. The bacteriology of this case was not worked out, but the association

of these two conditions reminds one of the cases found by Gordon in children. (L. G. B. Reports, No. 96 (New Series), 1914.)

Enteritis is probably fairly common, but was not often looked for. Injection of the mucous membrane of the colon was found in a fair number of cases, but records were not sufficiently kept to give figures on this point.

The work done by Captain W. Rolland, R.A.M.C., upon the bacteriology of this disease tends to show that the lesion most associated with the influenza bacillus is the haemorrhage, especially the haemorrhagic stage of pneumonia. When cultures were made from the infarct-like areas Pfeiffer's bacillus was found in nearly every case, and generally in almost pure culture. The same applied to the clinical aspect of the same condition. When the first haemoptysis was noticed, the influenza bacillus was easily recovered from the sputum.

Glands proved sterile on culture.

Effusions did not yield the bacillus—the usual organism in empyemata being the pneumococcus.

Blood cultures were always sterile.

Whatever the true bacteriology of influenza may be, there appears to be a fairly clear post-mortem picture associated with the activities of Pfeiffer's bacillus in this epidemic.

The most characteristic lesion is haemorrhage.

The most fatal lesion is probably myocarditis, and evidences of the extreme toxaemia are further to be found in the fatty liver and kidneys, and the enlarged spleen—and possibly meningeal oedema.

Enlargement of lymphatic glands was most marked in the thorax, where other toxins were at work in the lungs, so that this lesion can hardly be regarded as influenzal only.

Haemorrhage appears to take place in the lung, and in its immediate neighbourhood Pfeiffer's bacillus is found. Secondary infection leads to broncho-pneumonia which may be caused by a variety of organisms, prominent among which are the pneumococcus and a streptococcus. These are organisms commonly found in the respiratory passages, and multiply rapidly in the haemorrhagic pabulum the influenza bacillus has set free. Probably the influenza bacillus remains local, forming toxins which, carried over the body, produce the cardiac, hepatic, and renal lesions. No more than degenerative lesions are found elsewhere, such as a toxin, rather than an organism, would produce. The appearances described above may all be secondary to some other infection, but are nevertheless mainly caused by the influenza bacillus.

The type of pneumonia referred to as miliary pneumonia is due to extension of inflammation from the bronchioles into the neighbouring alveoli, and is not as a rule associated with haemorrhage. The pathology is probably not the same as in the more characteristically influenzal lesions.

7. REPORT ON PNEUMONIA FOLLOWING INFLUENZA

BY

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AND
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I. MATERIAL.

1. *Number of Cases.* 39 cases of severe pulmonary complications in the course of influenza.
2. *Classification of Cases.* 25 Lobar Pneumonia; 14 Broncho-pneumonia.
3. *Nationality.* 14 of the cases were British; 25 American.
4. *Source.* 12 field hospitals and casualty clearing stations.
5. *Season.* November 1918.

II. METHODS.

1. *Clinical.*
   (*a*) *Isolation* in a special ward as soon as pneumonia diagnosed. Beds separated by hanging sheets. White gowns and gauze face masks used.
   (*b*) *Blood Cultures.* Done as soon as possible after admission on nearly every case. 6–10 c.c. of blood in 50 c.c. 1 per cent. glucose bouillon.
   (*c*) *Sputa.* Examination: (1) Stained smears. (2) Attempting to isolate the pneumococcus by the Avery method.
2. *Post-mortem.* Examinations in all fatal cases.

III. LOBAR PNEUMONIA.

1. *Clinical Findings.*
   (*a*) *Mortality.* Of 25 cases, 17 died and 8 lived.
   (*b*) *Lobes consolidated—Surviving Cases* (8). Right lower lobe 5, both lower 1, right lower and right upper 1, both lower and right upper 1.

   Fatal cases (17). Right lower 1, left lower 4, both lower 6, both lower and right upper 1, both lower and left upper 1, left lower and left upper 1, right lower and right upper 1. In the two remaining cases there were signs of consolidation in one side, and diffuse broncho-pneumonic signs in the other.
   (*c*) *Temperature,* 102–105°.
   (*d*) *Recovery.* Crisis in one case. In seven lysis.
   (*e*) *Sputum.* Tenacious, blood tinged.
   (*f*) *Average Duration.* Fatal cases, 12 days; surviving, 9.

IV. Broncho-Pneumonia.

1. *Clinical Findings.*
   (*a*) *Mortality.* Of 14 cases, 11 died and 3 recovered.
   (*b*) *Lung Signs.* Great daily variations, often absence of pathological signs. Suppressed breathing at the bases, moist consonating rales, and patches of bronchial breathing in the interscapular spaces were common. In a few cases signs of consolidation in one or both lower lobes before death (the confluent cases).
   (*c*) *Temperature.* Great diurnal variations. Average lower than in lobar cases.
   (*d*) *Sputum.* Characteristic of influenza, pale-green, numular, with mucous *B. Influenzae.* In only 2 cases pneumococci grown.
   (*e*) *Average Duration.* Fatal cases, 15 days; surviving, 17.

V. Blood Cultures.

The Results are shown in Table I.

	Number Positive.	*Number Negative.*	*Per cent. Positive.*	*Per cent. Negative.*
Lobar Pneumonia	13	9	59	41
Broncho-Pneumonia	0	11	0	100

The pneumococcus was identified by the following characteristics:
(*a*) Greenish colour in blood bouillon.
(*b*) Diffuse cloudiness in bouillon with no flocculent precipitate and very little settling.
(*c*) Pneumococcus-like colonies on blood agar.
(*d*) Bile solubility (not always tested).
(*e*) Typing with sera furnished by the Rockefeller Institute. The types are shown in Table II.

	Type 1.	*Type* 2.	*Type* 4.
Number of Cases . . .	1	11	1

Table III.

Average number of days before death at time of cultures, and temperature at time of culture.

	Days.		*Temperature.*	
	Positive Culture.	*Negative. Culture.*	*Positive Culture.*	*Negative Culture.*
Lobar Pneumonia	2·9	1·5	103·6	104·5
Broncho-Pneumonia	0	3·7	—	102

Seven cases of lobar pneumonia in which blood cultures were negative or were not done were typed by isolating the pneumococcus from the sputum by the Avery method, or by culture obtained from lung puncture. These showed four cases of type 2, and three of type 4 pneumococcus. In 20 cases of lobar pneumonia, therefore, the types of pneumococcus occurred as follows: type 1, one case; type 2, fifteen cases; type 4, four cases. In no case was type 3 recovered.

In cases having broncho-pneumonia the pneumococcus was isolated from the sputum in two cases only, one type 2 and one type 4—both fatal cases. Repeated attempts to grow the pneumo-

coccus in the other cases failed. Often few or none of these organisms could be seen in stained spreads of the sputum. The bacillus of influenza was found in the sputum of all the cases of broncho-pneumonia.

TABLE IV.

Mortality with reference to Positive Blood Cultures.

	Of Cases with Positive Cultures.		*Of Cases with Negative Cultures.*	
	Number.	*Per cent.*	*Number.*	*Per cent.*
Living . . .	1	7·7	6	66
Dying . . .	12	92·3	3	33

VI. PATHOLOGY.

Pathological Findings. The gross appearance of pneumonic lungs suggests a division into the following groups :

(1) *Pure broncho-pneumonia.* The lesions are discrete with definite limits. Pleurisy is not often concurrent.

(2) *Rapidly spreading broncho-pneumonia.* The patches are large and ill-defined, often becoming confluent. Even when a whole lobe is involved in a confluent process, it does not have the massive appearance of true lobar pneumonia and an unevenness is evident on palpation and section. Usually there is no pleurisy ; if present it is of the dry kind. Two cases in our series were of this type.

(3) *Pure lobar pneumonia.* The lungs are typical. They are large, massive, and evenly firm on palpation. In distinction to the two previous types there is almost invariably a marked fibrino-purulent pleurisy over the affected lobe. This pleural lesion is lacking over other lobes in the same case, which are not involved in the lobar process, even though they have marked broncho-pneumonia.

The cut surface shows even consolidation, some in the stage of red hepatization, some in the grey stage.

(4) In a certain number of cases the lobar pneumonias give evidence of a previous broncho-pneumonic process in the same lobe. Immediately about the bronchi there are areas showing older, harder lesions, of a red or grey colour.

(5) In many cases the lobes not involved in the lobar process show a broncho-pneumonic process. It is quite probable that in some of these cases there is also a broncho-pneumonic process in the lobe with lobar pneumonia, which is obscured by the lobar consolidation.

(6) In three cases a lobe or lobes are oedematous, with red fluid, and airless, but not consolidated.

Associated processes. Marked bronchitis is nearly always present. Pericarditis is noted in three cases. In one case it is of the dry adhesive type, and concurrent with broncho-pneumonia. In the two other cases it is purulent and associated with lobar pneumonia. The pericardial exudate contains a pure culture of pneumococcus in each of the two latter cases. In one case the pneumococcus, on typing, proves to be type 2, the same type as in the blood culture and lung culture.

Empyema. In four cases empyema occurred with lobar pneumonia, in one case with broncho-pneumonia. Marked collapse occurred only in the broncho-pneumonic case.

Pleural exudate. In six cases, all lobar pneumonia, there was excess of pleural fluid, slightly cloudy, and reddish-brown.

8. ON THE AGGLUTINATION OF *B. INFLUENZAE* (PFEIFFER) BY THE SERUM OF PATIENTS SUFFERING FROM INFLUENZA.

By Captain P. Hartley, R.A.M.C., T.F.

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During the recent epidemic, at the end of 1918, the opportunity occurred for testing the sera of patients who were suffering from influenza for agglutinins to *B. influenzae*. It was thought that investigations on these lines might throw some light on the vexed and still unsettled question of the part which Pfeiffer's bacillus of influenza plays in the disease. The epidemic subsided rather quickly in this Base and in consequence some projected experiments were left unfinished. The results which have been obtained up to the present are set out briefly below.

I. Material Used.

The first material which became available was serum obtained from the heart blood taken at autopsy on cases of influenza. In all, twenty-one satisfactory samples of serum were obtained and examined. They were supplied by Major S. W. Patterson, R.A.M.C., Pathologist, No. 5 General Hospital, who, with Little and Williams of this hospital, was investigating at the time a series of cases of influenza. These workers investigated fifty-six cases and demonstrated the presence of *B. influenzae* (Pfeiffer) in the lung or bronchus of every case except one. The next material studied consisted of serum from patients who ultimately recovered from the disease. It was hoped to be able to compare the agglutinin content of the serum of the same patient at different stages of the disease, but on account of lack of material this was only possible in one case.

II. Method.

A macroscopic method was used. The patient's serum was tested at dilutions of 1 in 50, 1 in 100, and 1 in 200 only. Equal volumes of diluted patient's serum and emulsion of *B. influenzae* were mixed in a small test tube, heated to 55° C. for three hours and read off from fifteen to thirty minutes after removal from the bath. The racks were left on the bench until the following morning when a second reading was made. In each experiment, when a number of patients' sera were being tested, at least three and sometimes five samples of normal sera were set up and tested in precisely the same way.

III. The Emulsions of *B. Influenzae* Used.

As some unexpected difficulties were encountered in the earlier experiments the following notes may be of interest, and perhaps of some use, to other workers on this subject.

Eight strains of *B. influenzae* were used ; seven had been isolated from the lung and one from sputum. In the earlier experiments the organism was grown on unheated rabbit's blood-agar, on plates which were inoculated from a pure culture by making a series of parallel strokes. The plates were incubated for eighteen to twenty-four hours at 37° C., a number of colonies picked off and examined, and if found satisfactory the growth was taken off with a platinum loop and emulsified in a measured quantity of saline and diluted if necessary. A unit volume of this emulsion was then heated to 55° C. for three hours, and if the suspension remained perfectly uniform and unagglutinated the tests with diluted patient's serum were proceeded with.

These precautions are necessary as it was found that different strains of *B. influenzae* exhibit very varying degrees of 'auto-agglutinability'. Some strains agglutinate after standing on the bench for about an hour, others are stable at room temperature but agglutinate after heating at 55° C. for three hours, while others remain perfectly stable after heating at 55° C. even for twenty-four hours. Moreover, it was found that a strain of *B. influenzae* may yield a perfectly stable and satisfactory emulsion on one day, yet a few days later another generation of the same strain may yield an emulsion which agglutinates spontaneously at room temperature (strains 40 and 22 behaved in this way). The following observation was also made : A culture of strain 22 growing on unheated rabbit's blood-agar was examined and found to be typical. From this parent slope subcultures were made on two plates, one consisting of unheated rabbit's blood-agar and the other of heated (70° C.) rabbit's blood-agar. After incubation at 37° C. for eighteen hours each plate showed a good growth, but in neither case was the organism quite typical. The culture on the unheated rabbit's blood-agar showed long filamentous forms, while that on the heated rabbit's blood-agar yielded organisms which were a little longer than usual but otherwise typical. In the former case it was found to be very difficult to emulsify the growth in saline and the emulsion when made agglutinated on standing on the bench. In the latter case the growth emulsified easily, but spontaneous agglutination occurred at room temperature. Earlier in its history this strain had yielded emulsions which were perfectly stable, and since the above observation was made it has returned to normal habits.

At the time it was thought that the concentration of sodium chloride in which the bacilli were suspended was a factor affecting their agglutinability, but experiments showed that this was not the explanation. As a matter of convenience and routine the agglutination experiments described in this paper have all been carried out in a solution in which the concentration of sodium chloride was always the same in every tube, namely 0·5 per cent.

As a result of the later experiments, and other work on the bacillus of influenza, the very distinct impression has been gained that the blood used in making the media is a factor which affects the auto-agglutination of the emulsion as well as the morphological character of the organism. When using unheated blood one could never tell whether the resulting emulsion would serve for experimental purposes

or not. The emulsion had to be made, examined, and tested every time.

In the later experiments Matthew's medium was used and this was found to be perfectly satisfactory. Luxuriant growths were invariably obtained and these yielded stable emulsions of morphologically typical organisms.

IV. Summary of Results.

Series A. *Examination of twenty-one Samples of Serum from the Heart Blood of Fatal Cases of Influenza.*

In most cases the same sample of serum was tested against several strains of *B. influenzae* either on the same, or different, days.

Two readings of the tubes were made; the first from fifteen to thirty minutes after removal from the bath (the immediate reading), and the second on the following morning (the late reading). It was found that agglutination was sometimes observed after eighteen to twenty-four hours where none had been visible immediately after removal from the bath. 'Naked eye' agglutination was looked for throughout, no hand lens being used. In no case did any sample of normal serum agglutinate any strain of *B. influenzae* used, and all strains were tested before use for auto-agglutination and put up again with the sera during the tests. No strain used exhibited auto-agglutination.

The results obtained are set out below. The numbers refer to serial numbers given to patient's serum or to the strain used. The entry 'late' indicates that agglutination was not apparent after heating for three hours at 55° C., but was easily visible to the naked eye after standing at room temperature overnight.

Table I.

Patient's serum tested.	*Duration of illness.*	*Strains of B. influenzae tested.* (*a*) *Strains agglutinated by patient's serum.*	(*b*) *Strains not agglutinated by patient's serum.*
41	12 days	'Legge,' 'Hudson' (late), 40, 24, 45, 22, and 63	
42	6 ,,	'Legge,' 'Hudson' (late), 40, 24, 45, 22.	
53	11 ,,	'Legge,' 'Hudson' (late), 40, 22	
6	6 ,,	'Hudson,' 22, 40 (late), 63, 64	
8	8 ,,	'Hudson,' 24, 22	45
39	8 ,,	'Hudson,' 22, 63	40, 64
Freeman	? ,,	40, 63, 64	
35	17 ,,	40, 63, 64	
3	15 ,,	40, 63, 64	
40	10 ,,	'Legge,' 40	'Hudson'
52	10 ,,	24, 22	45, 40, 63, 64
7	11 ,,	63	
51	10 ,,	40 (late)	
25	8 ,,	'Hudson'	
36	8 ,,	63 (late)	40
34	13 ,,		40, 64
26	12 ,,		40
55	13 ,,		64
5	9 ,,		64
23	16 ,,		24, 45
24	10 ,,		24, 45

Table II.

The degree of agglutination is given in the two following summaries.

(*a*) *Immediate reading.*

Of the 21 sera examined,
7 agglutinated one or more strains of *B. influenzae* at 1/200.
5 „ „ „ „ „ „ „ „ 1/100.
1 „ „ „ „ „ „ „ „ 1/50.
8 failed to agglutinate the strains of *B. influenzae* against which they were tested.

(*b*) *Late reading.*

Of the 21 sera examined,
10 agglutinated one or more strains of *B. influenzae* at 1/200.
4 „ „ „ „ „ „ „ „ 1/100.
1 „ „ „ „ „ „ „ „ 1/50.
6 failed to agglutinate the strains of *B. influenzae* against which they were tested.

Table III.

Summary showing the Agglutination obtained with the Strains used.

Strains of B. influenzae used.	*No. of samples of sera against which tested.*	*No. of cases which gave* (*a*) *Agglutination.*	(*b*) *No agglutination.*
'Legge'	4 cases	4	None
22	7 „	7	„
'Hudson'	8 „	7	1
63	9 „	8	1
24	6 „	4	2
45	6 „	2	4
40	14 „	9	5
64	9 „	4	5

Thus, nearly two-thirds of the sera tested agglutinated *B. influenzae* at a dilution of at least 1 in 50, and one-third of the cases agglutinated the bacillus at the relatively high dilution of 1 in 200. Unfortunately these were not tested out to ascertain their end point of agglutination.

The average duration of illness in this series was ten days.

Ten cases agglutinated all strains against which they were tested, and six failed to agglutinate any strain at the lowest dilution tested, viz. 1 in 50. Five sera agglutinated some strains and failed with others. This is an interesting point, and whether it indicates that there are serologically different types of *B. influenzae* is a matter for further investigation.

Series B. *The Agglutinin Content of the Serum of twenty Patients who ultimately recovered from Influenza.*

The average duration of illness in this series of twenty cases was seventeen days. Ten of the sera were tested against one strain of *B. influenzae* only, six were tested against two strains, and four against three strains.

Table IV.

(*a*) *Immediate reading.*

Of the 20 sera examined,
3 agglutinated one or more strains of *B. influenzae* at 1/200.
4 „ „ „ „ „ „ „ „ 1/100.
6 „ „ „ „ „ „ „ „ 1/50.
7 failed to agglutinate any strain of *B. influenzae* against which they were tested (4 of these 7 were tested against 2 strains, and 3 against 1 strain only).

(*b*) *Late reading.*

Of the 20 sera examined,
4 agglutinated one or more strains of *B. influenzae* at 1/200.
6 „ „ „ „ „ „ „ „ 1/100.
4 „ „ „ „ „ „ „ „ 1/50.
6 failed to agglutinate any strain of *B. influenzae* against which they were tested (4 of these 6 were tested against 2 strains and 2 against 1 strain only).

Comparing the two series A and B, although the numbers of sera tested are not great, the number of times that agglutination was observed was about the same in the two series, but agglutination occurred at a higher dilution in series A than in series B. In this connexion it is interesting to note that the serum was taken, on an average, ten days from the onset in series A, and, on an average, seventeen days from the onset in the case of series B.

Series C.

Unfortunately there is, as yet, only one completed observation in this series, the object of which was to study the agglutinin curve to *B. influenzae* in patients suffering from influenza.

In this case the first sample of serum was taken on the sixth day of the disease, and this agglutinated *B. influenzae* completely at 1 in 200 (immediate reading); a second sample was taken on the tenth day of the disease, and this agglutinated *B. influenzae* at 1 in 100 (immediate reading); a third sample was taken on the fifteenth day, and no agglutination was detectable by the naked eye at 1 in 50 at the immediate reading, but by next morning agglutination had occurred at this dilution. In making these agglutination tests the same strain of *B. influenzae* was used throughout.

V. Conclusions.

(i) The sera from forty-two cases were examined for the presence of agglutinins to *B. influenzae*, and it was found that twenty-seven of these agglutinated the bacillus at a dilution of at least 1 in 50.

(ii) The serum of normal healthy persons did not agglutinate the emulsions of *B. influenzae* used at a dilution of 1 in 50.

(iii) Evidence was obtained that agglutinins to *B. influenzae* are developed at an early stage of the disease and that there is a rapid decrease of these agglutinins during convalescence (cf. Fleming, *Lancet*, London, 1919, i. 139).

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